ABSTRACT OF THESIS

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Title of Thesis The Influence of Physical and Pharmacological Factors on the Growth and Behaviour of Neoplasms.

The Brown-Pearce carcinoma was chosen as a convenient experimental tumour for investigating the influence of physical factors on the behaviour of neoplasms and particularly for understanding the mechanism of tumour necrosis. The rapidity of the growth and the type of the vasculature in this tumour were important factors considered in planning the experiments that form the first part of the thesis; namely, hypotension is a factor primarily concerned with necrosis in this and possibly in other malignant tumours of similar vascular morphology.

The pressure developed in the tumour (tissue pressure) was quantitatively studied. A "manometric method" was used to estimate this pressure in normal testicular tissue and in Brown-Pearce carcinoma implanted and grown in the contralateral testis; the estimations were carried out in normotensive rabbits and after induction of hypotension by pharmacological means. The methods were reviewed and evaluated. It was found that in spite of the arguments raised about its validity, manometry served as a satisfactory and reliable method for this particular project when relative rather than absolute values are required. The tissue pressure measured in this way was defined as the pressure exerted from all directions which tends to obliterate collapsible structures such as the peripheral parts of tubules and vessels, or thin-walled capillaries; therefore, it was named appropriately the total tissue pressure (TTP).

A suggestion was made that the increase of TTP of the tumour with the lapse of time could explain part of the mechanism which causes necrosis in this and possibly in other tumours by compression of the tumour blood vessels.

A group of experiments was designed to study the effect of hypotension, induced by Ismelin injections, on the origin of necrosis in Brown-Pearce carcinoma. The experiments were carefully timed to exclude the complication of spontaneous necrosis which is common in this tumour after the third week of implantation. Hypotension induced and maintained for periods of 48 hours in rabbits bearing Brown-Pearce carcinoma intratesticularly produced necrosis far exceeding that in the controls. Through these experiments it was possible to confirm that hypotension is an important primary cause of cell death in the Brown-Pearce carcinoma.

Detailed study on the relationship between the vascularity and cell death in the tumour was made and the opinions of the previous workers on the subject discussed. Two staining techniques were used to investigate the tumour vasculature. By luxol-fast blue it was possible to demonstrate dilated vessels in 4μ sections of Brown-Pearce carcinoma under physiological conditions. The necrotic tumour was found to have many dilated vessels packed with red cells; therefore, the inference can be drawn that cell death in this tumour was not due to absence of capillaries and blood vessels. Moreover, high vascularity was seen in some necrotic regions.

Another staining technique, namely the picro-Mallory stain was used to study the physical state of the tumour vessels indirectly by demonstrating in histological sections vessels that contain static or flowing blood. Fibrin deposition indicating stasis was frequently noted in the dilated vessels of the necrotic tumour but

Use other side if necessary.

not in the non-necrotic regions. It is suggested that the sequence begins with hypotension which alters the relationship of intra- and extravascular pressure: a relative increase in extravascular pressure leads to compression of tumour vessels with consequent dilatation and stasis, though no deduction could be made as to the precise time when the circulation had stopped.

Therefore, in this part of the thesis, evidence is presented that the viability of the tumour cells is predominantly influenced by physical forces in the tissues capable of maintaining a high hydrostatic pressure in the tumour circulation necessary for a uniform and adequate blood supply, a feature which to a large extent depends on the type of vasculature in that particular tumour. Far more important is the demonstration that by alteration of a purely physical factor (induction of hypotension) tumour necrosis has occurred.

Part 2 is a histological study of the growth characteristics of ninety cases of breast biopsies received in this Department. Of those, forty-five from women known to have taken oral hormonal contraceptives, and sections from this group have been compared with those of forty-five age-matched controls with no history of hormonal therapy. The incidence of fibroadenoma, fibrocystic disease and carcinoma was studied and the main microscopic features were described and compared. Epitheliosis was a prominent feature in the breasts of women taking hormonal therapy. A case of marked epitheliosis with papillomatosis was described; the lack of cellular atypia and invasion of the basement membrane excluded the diagnosis of malignancy. The possibility of carcinoma <u>in situ</u> in some of the cases was raised. The relationship between epitheliosis and carcinoma and the significance of certain changes in the breast provoked by these hormones were discussed.

THE INFLUENCE OF PHYSICAL AND PHARMACOLOGICAL FACTORS ON THE GROWTH AND BEHAVIOUR OF NEOPLASMS

by

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CONTENTS (contd.)

PREFACE

This work in two parts is a study of the influence of certain physical and pharmacological (oral contraceptive hormones) factors on the growth and behaviour of neoplasms.

The first part, which is an experimental study, is an attempt to investigate the relationship between hypotension and necrosis in a neoplasm under standard conditions of growth. The Brown-Pearce carcinoma as an experimental tumour model was used for this purpose.

The second part which is a histological study concerns the structural changes in breasts in women taking the "contraceptive pill", from whom biopsies were received in this Department. This part of my work was suggested by my supervisors, Dr. A. A. Shivas and Professor G. L. Montgomery, during the time when I was waiting for the experimental studies to begin. The subject has become of interest in recent years mainly because of the concern that a possible relationship might exist between the hormone intake and the abnormal morphology of the breast.

Although the two parts may seem superficially to have no direct connection, in actual fact they are complementary aspects of the problems of tumour growth and behaviour.

ACKNOWLEDGEMENTS

The work of this thesis was carried out in the Department of Pathology, University of Edinburgh, and I am deeply grateful to Professor G. L. Montgomery for giving me the opportunity to work in his Department. The research was directed by him and to some extent by Dr A. A. Shivas whom I should like to thank. On the technical side, I should like to thank Mr J. Waugh, the Chief Technician; Mr W. Robb, the Animal House Technician; Mrs. S. Wilson and Mrs D Banks.

Dr Shivas resigned from my supervision before this work was completed, and I carried out the writing of the thesis under the direction of Professor G. L. Montgomery who then had to leave the University before my work was completed. Dr A. E. Stuart, Reader and Acting Head of the Department kindly agreed to take responsibility during the time necessary for revision of the thesis, and I am very grateful to him for his capable direction and tutelage.

Finally, I should like to thank Calouste Gulbenkian Foundation for being one of its scholars.

SUMMARY

The Brown-Pearce carcinoma was chosen as a convenient experimental tumour for investigating the influence of physical factors on the behaviour of neoplasms and particularly for understanding the mechanism of tumour necrosis. The rapidity of the growth and the type of the vasculature in this tumour were important factors considered in planning the experiments that form the first part of the thesis; namely, hypotension is a factor primarily concerned with necrosis in this and possibly in other malignant tumours of similar vascular morphology.

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Therefore, in this part of the thesis, evidence is presented that the viability of the tumour cells is predominantly influenced by physical forces in the tissues capable of maintaining a high hydrostatic pressure in the tumour circulation necessary for a uniform and adequate blood supply, a feature which to a large extent depends on the type of vasculature in that particular tumour. Far more important is the demonstration that by alteration of a purely physical factor (induction of hypotension) tumour necrosis has occurred.

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PART I

AN EXPERIMENTAL STUDY OF THE RELATIONSHIP BETWEEN PHYSICAL FACTORS IN THE TISSUES AND NECROSIS IN BROWN-PEARCE CARCINOMA

CHAPTER 1

1. Introduction

The purpose of research on experimental tumours is to extend our knowledge on tumours in general, and to provide certain facts which might be useful in clinical practice.

This part of the thesis deals with experimental studies and is an attempt to investigate the hypothesis that induced systemic hypotension may produce necrosis in a neoplasm under standard conditions of growth. The hypothesis stems directly from experiments undertaken by A. A. Shivas and indirectly from the earlier observations of J. S. Young on the significance of relative intra- and extra-vascular premises in the growth and spread of tumours.

In 1950 Young and Griffith published an important paper entitled "The dynamics of parenchymatous embolism in relation to the dissemination of malignant tumours". The basic theorem which these authors set out to examine was a simple problem in differential pressures. They postulated that fragments of tissue (normal or neoplastic) would be prevented from entering the lumina of vessels if the intraluminal pressure exceeded the extraluminal pressure. Conversely, extra-vascular pressure greater than the intraluminal would favour the entrance of particles and their subsequent transport provided that the relatively high extravascular pressure did not collapse the thin-walled vessels and sinusoids through which transit is known to occur. They constructed an elegant hydrostatic model in which a compressible rubber tube could be subjected to varying external and internal fluid pressures at will. The importance of differential pressures was clearly shown and it was demonstrated conclusively that

bodies (e.g. polystyrene beads) suspended in a fluid or semisolid medium cannot enter the lumen of a tube by holes in its wall so long as the intraluminal pressure is greater than the external pressure, but they can and do enter the lumen when the external pressure is the greater.

This fundamental paper was a major contribution which continues to stimulate thought, especially in the influence of physical factors in tumour growth and in the development of metastases, a field in which Shivas has been particularly active. At this stage it is sufficient to summarise his relevant experiments. He noted (1959) that Brown-Pearce carcinoma, implanted and growing vigorously in the cerebral hemispheres of rabbits for up to 32 days failed to produce metastases, most probably because of a high intravascular pressure which maintains the patency of the tumour vessels.

Subsequently, Shivas with Finlayson (1965) investigated the resistance of arteries to invasion by tumour. Using rabbits, their technique involved the exposure of approximately 1.5 cm. of femoral artery and its isolation between double ligatures (a procedure which does not impair the viability of the arterial wall since the vasa vasorum are not disturbed) and the implantation of fresh fragments of Brown-Pearce carcinoma close to the collapsed arterial wall between the ligatures. Controls were provided by inserting similar implants against the unligated artery of the other limb. The result showed

that when the femoral arteries of rabbits are deprived of their blood content and thus of their intraluminal pressure, their walls are actively invaded and destroyed by Brown-Pearce carcinoma. Thus the authors concluded that the normal resistance of arteries to invasion by malignant tumours depends entirely on their intraluminal pressure. In their discussion, the authors emphasise the apparently peculiar nature of the circulation within the tumour and remark "There is clearly scope for further work in this field and on the still more fundamental question of the nature of the mechanism responsible for the development and maintenance of tumour circulation".

Hence the stimulus to the work which forms the basis of this part of my thesis.

2. Plan of the Investigation

The essentials were, an experimental model, a method of inducing controlled hypotension, of estimating systemic blood pressure in rabbits, of measuring tissue pressure in the tumour and the normal tissue and of studying relationship between hypotension and necrosis in the tumour.

Experimental Model

The Brown-Pearce carcinoma growing intratesticularly in rabbits was chosen for several reasons. It is a primary malignant epithelial tumour discovered by Brown and Pearce (1920-21) during their experimental investigation of syphilis in rabbits. The tumour had grown on the site of a healed scrotal chancre in a rabbit four years after the animal had been inoculated with the Zinsser-Hopkins strain of Treponema pallidum, and the tumour led to the death of the rabbit within a few months. The second generation of rabbits inoculated with the tumour were cleared of syphilis by arsphenamine treatment. Brown-Pearce carcinoma has been propagated continuously in laboratories throughout the world for many generations in rabbits and its biological behaviour fully documented. After intratesticular inoculation it grows slowly for a few days and then rapidly, so that after the first week it is palpable and may attain 2-3 cm. in diameter during the third week. By the end of the fourth week the whole body of the testis is usually replaced by tumour. Focal necrosis may be seen in the third week, but massive spontaneous necrosis rarely occurs earlier than the fourth week. Extension of the tumour takes place directly by the

spermatic cord and subsequently by blood and lymph vessels. The strain used in the experiments recorded here had been maintained in this laboratory for several years. Its behaviour with regard to necrosis was known and it was assumed that by completing the experiments before the twenty-first day, spontaneous massive necrosis would not complicate the results. Thus, in addition to its availability, the biological behaviour of the Brown-Pearce carcinoma grown intratesticularly, favoured its choice for this work.

Figures 1-3 illustrate the histology of the Brown-Pearce carcinoma.

3. Tumour Transplantation

The standard method of intratesticular inoculation of small fragments of Brown-Pearce carcinoma using standard lumbar puncture needles was used throughout the experiments. Care was exercised in the selection of a tumour which was actively growing and free from necrotic tissue. As a rule a tumour of about three weeks' duration was used in each series of transplant. The donor animal was killed by a large intravenous dose of nembutal (60 mg./kg., body weight) and the testis removed aspetically. The tumour was dissected and the parts which appeared to have grown most actively placed in a Petri dish containing normal saline, and finely-cut with a pair of scalpels into small fragments. Using standard lumbar puncture needles these fragments were inoculated into the testicular parenchyma of rabbits, each rabbit receiving one fragment in the testis. It has been found that under normal conditions, good growth was obtained from this method of implantation within 10-21 days.

4. Blood Pressure Determination in Rabbits

Apparatus and Technique

The blood pressure in the rabbits was recorded from the central artery of the ear, using the Grant (ear capsule). This method is simple and if carefully carried out gives fairly accurate and reproducible results with minimal disturbance of animals. The apparatus consists of a sphygmomanometer connected to a metal capsule by a rubber tube. The metal capsule is cylindrical and measures about 1.5 cm. in diameter. At one end it is covered by a transparent window and at the other, by a distensible transparent membrane. The metal capsule is carried on a metal ear holder. The connection should be air-tight. The blood pressure recorded by this method therefore, is the minimum pressure which causes a temporary occlusion of the central artery of the ear with arrest of blood-flow in the vessel. The apparatus is shown diagramatically in Figure 4.

5. Experimental Hypotension in the Rabbit

Administration of hypotensive drugs has been widely practised experimentally in animals, but in many cases the results are not satisfactory.

Guanethidine Sulphate (Ismelin)

The choice of Ismelin as a hypotensive agent in rabbits has been found to be satisfactory in my experience for the study of the behaviour of Brown-Pearce carcinoma in a hypotensive environment. It is an adrenergic neurone-blocking drug which acts by selectively blocking transmission in the post-ganglionic adrenergic nerves, thus preventing release of adrenaline and nor-adrenaline from the nerve endings.

So far as I am aware, the use of Ismelin to produce hypotension in tumour-bearing animals has not previously been reported in the literature. Ismelin has certain important properties that make its application in this research project useful:-

1. It does not have the disadvantages of parasympathetic block.
2. It has a prolonged action which is not liable to produce tolerance.
3. It causes a reduction in cardiac output and reduces vaso-constriction.
4. Transient side-effects were easily controlled in these experiments.
5. It can be given by intravenous injection safely in pharmacological doses.
6. It is widely used in clinical practice.

The pharmacological effects of guanethidine can be demonstrated in cats. After intravenous injection into an anaesthetised cat, the first effects are sympathomimetic, producing a rise of the blood pressure and acceleration of the heart. Somewhat later, the signs of adrenergic neurone-blocking became apparent, and the blood pressure began to fall (Boura and Green, 1965). Maxwell *et al.* (1960) found that guanethidine lowers arterial pressure and reduces the cardiac index and pulse pressure in dogs. Cohn *et al.* (1963) found that the transient pressor response following intravenous administration of guanethidine in man is of much shorter duration than that seen in animals, perhaps owing to the considerably smaller doses used in these human studies. Altura and Zweifach (1966) studied the influence of reserpine and guanethidine on the vascular reactivity of micro-circulation in rats. They observed that within two minutes after the intravenous injection of 5 mg./kg. of guanethidine into the rat, the mesacaecal microcirculation exhibited arteriolar dilatation together with venular constriction, and after repeated administration of guanethidine intramuscularly, the effect of arteriolar dilatation still persists. Vasodilatation in rabbit ear vessels has been observed after guanethidine injection (Holton and Rand, 1962).

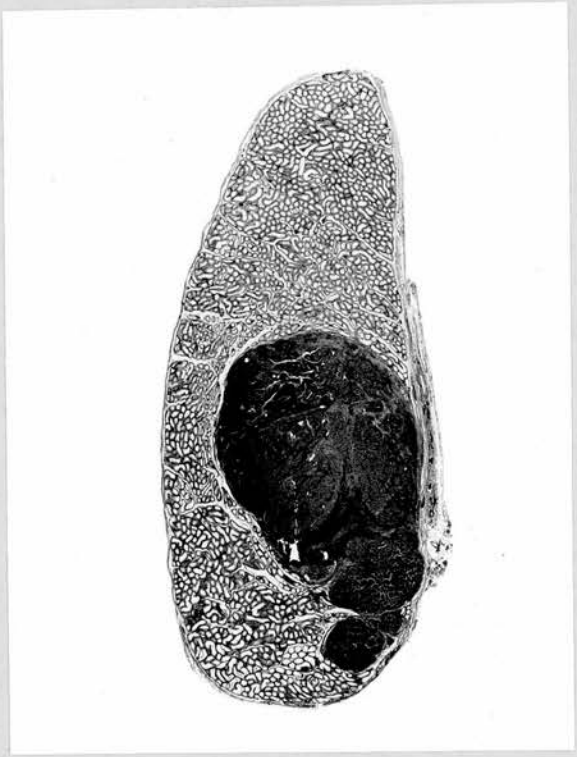


Fig. 1 Brown-Pearce carcinoma, 18 days after implantation in rabbit testis.

H. and E. x 4.

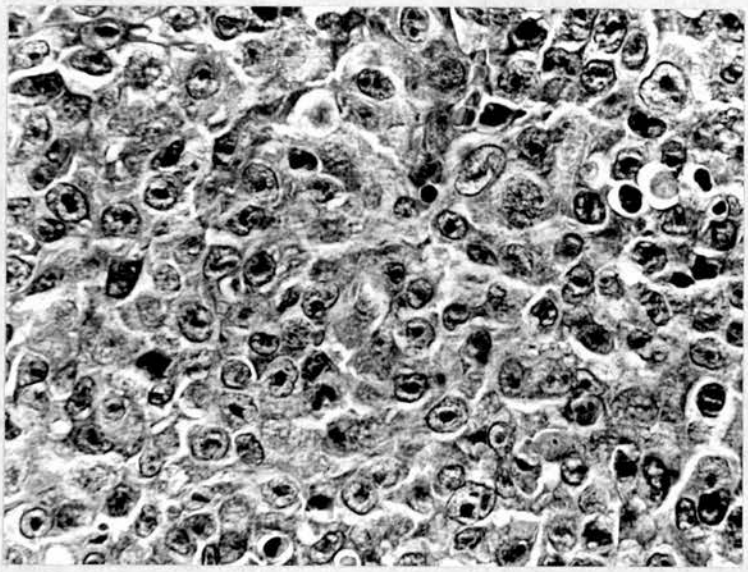


Fig. 2 High power view of Fig. 1. Note the cells are closely packed with little stroma.

H. and E. x 475

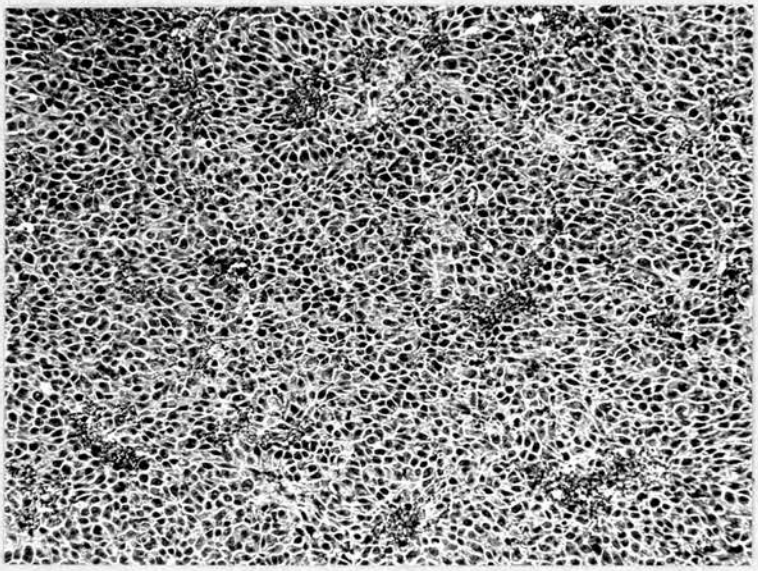


Fig. 3 Brown-Pearce carcinoma. Note the highly cellular tumour with many intact capillaries and minimal necrosis.

H. and E. x 110.

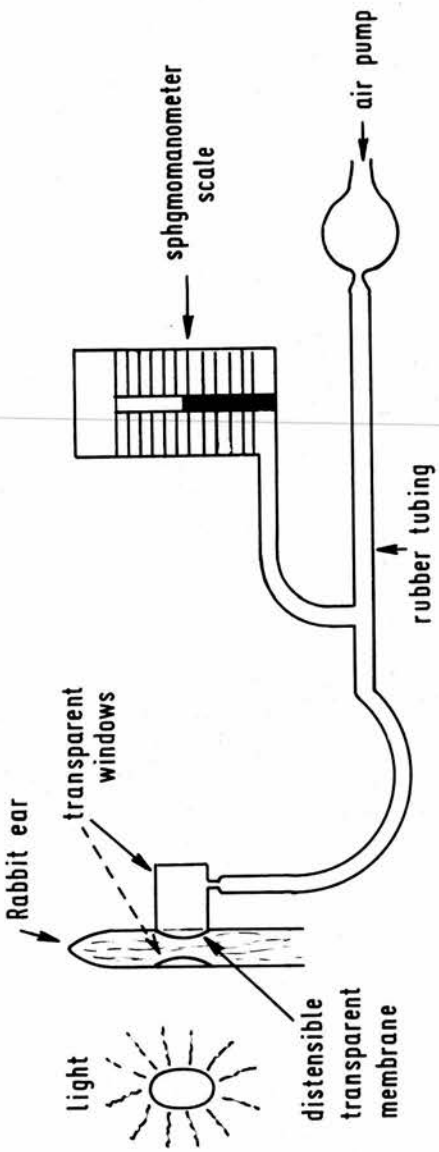


Figure 4. GRANT CAPSULE APPARATUS FOR ESTIMATION OF BLOOD PRESSURE

CHAPTER 2

Experimental Work

Experiment 1

To study the effect of Ismelin on rabbits and to establish a suitable dosage for controlled hypotension.

The object of this initial experiment was to determine the normal peripheral blood pressure in the rabbit, to work out a standard dose of Ismelin which could be used satisfactorily in this project and to study the effect of smaller (test) and larger (pharmacological) doses of the drug on the blood pressure of rabbits.

Material and Methods

Five healthy male rabbits of mixed strain and of weights 2.5 - 3.0 kg. were used. The blood pressure was determined for each animal from the central artery of the ear using the Grant capsule. The first group of two animals were given test doses of Ismelin intravenously of 0.2 mg./kg. body weight, and the second group of three animals larger (not lethal) doses of Ismelin intravenously of 10 mg./kg. body weight. The blood pressure was recorded every six hours over a period of forty-eight hours.

Results

The results are summarised in Tables 1 and 2 and Text Figures 1 and 2.

Comment and Conclusions

The "test dose" calculated according to the animal body weight of 0.1 - 0.2 mg./kg. was found to be unsatisfactory for the purpose of

maintaining an adequate drop in blood pressure over a sufficiently long period. For this reason a "pharmacological dose" was worked out which can produce a sufficient degree of hypotension suitable for these investigations. A "pharmacological dose" by definition is the minimum intravenous dose of Ismelin capable of inducing an adequate fall of the blood pressure in the rabbit and maintaining this hypotensive state over a prolonged time without producing any marked pathological changes in the animal. This dose was found to be 10 mg./kg. body weight every twenty-four hours. Therefore, the conclusion drawn from this experiment is that the intravenous administration of Ismelin in pharmacological doses produces a satisfactory fall of the blood pressure in the rabbits over a relatively long period of time, and thus can be used as a pharmacological means for inducing hypotension in rabbits.

TABLE 1

Effect of Small Doses of I.V. Ismelin on the Blood Pressure of Experimental Rabbits

1. Doses of Ismelin	Time of Estimation	2. Blood Pressure Estimations	
		Animal 1	Animal 2
0.2	10.00 a.m.	120	110
	10.05 a.m.	110	110
	10.30 a.m.	80	80
	4.00 p.m.	95	95
	10.00 p.m.	110	105
0.2	10.00 a.m.	120	110
	10.30 a.m.	85	80
	4.00 p.m.	95	90
	10.00 p.m.	115	105
	10.00 a.m.	120	110

1. Doses of I.V. Ismelin in mg./kg./24 hours.

2. Blood pressure in mm.Hg.

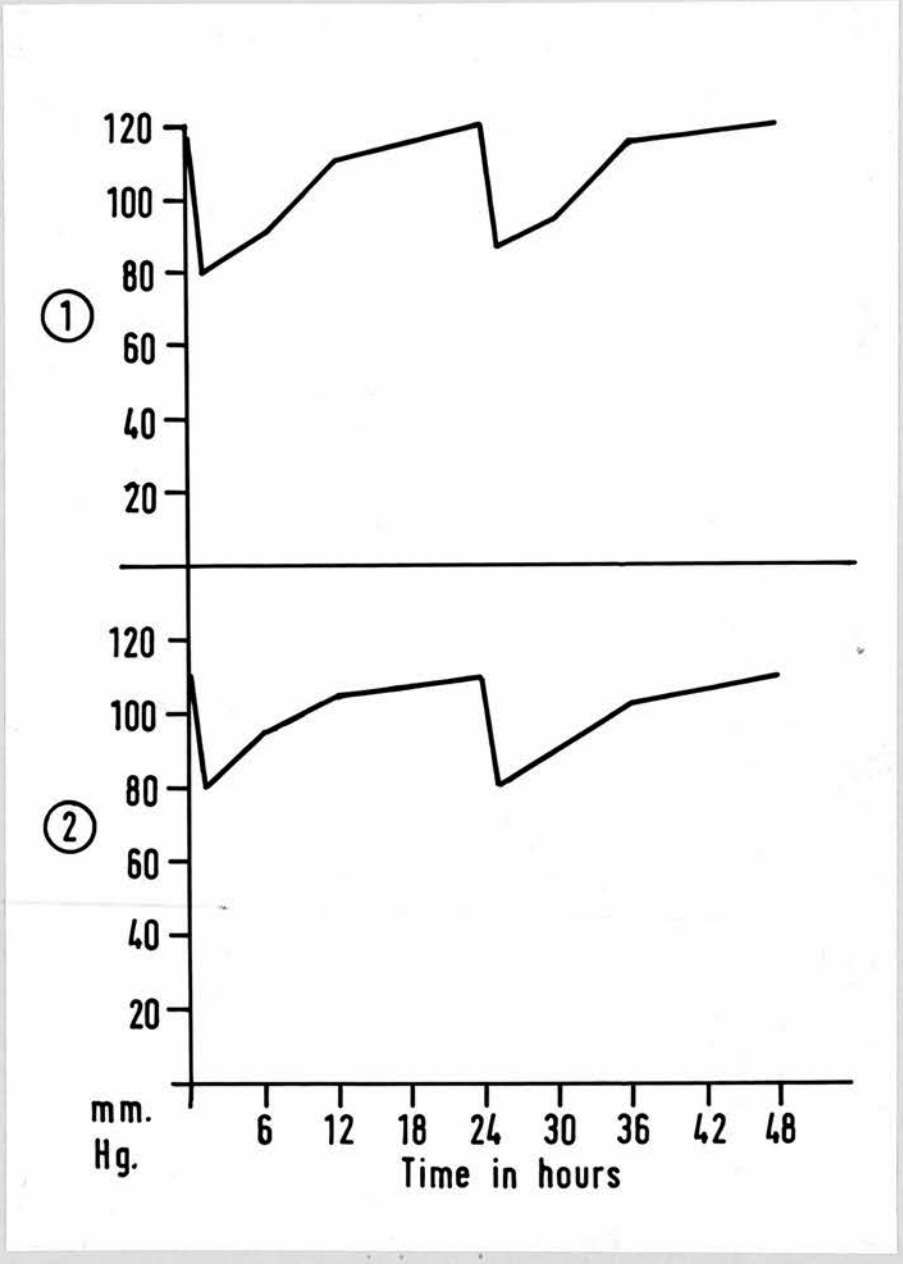
TABLE 2

Effect of Larger Doses (not lethal) of I.V. Ismelin on the Blood Pressure of Experimental Rabbits

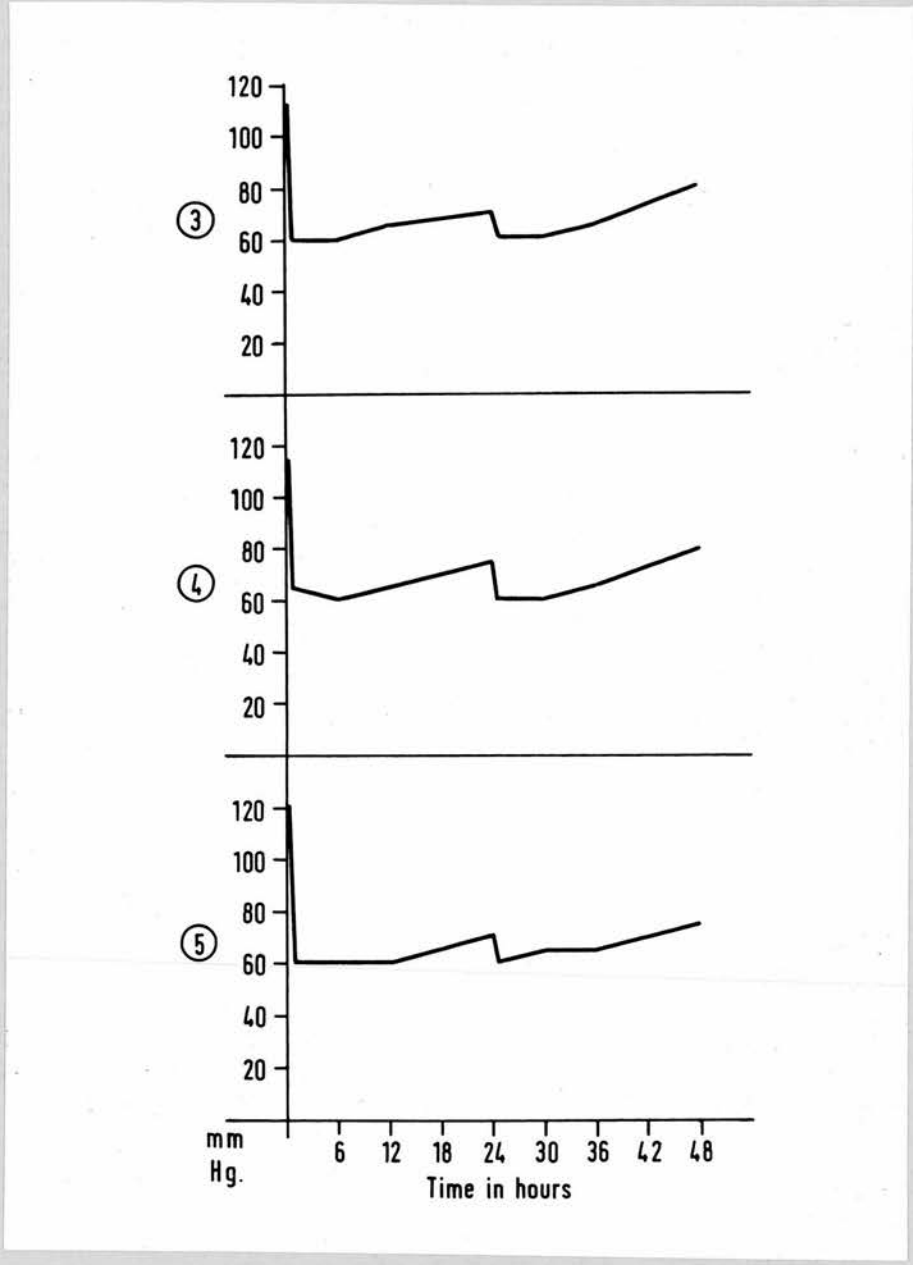
1. Doses of Ismelin	Time of Estimation	2. Blood Pressure Estimations		
		Animal 3	Animal 4	Animal 5
10	10.00 a.m.	110	115	120
	10.05 a.m.	115	115	125
	10.30 a.m.	60	65	60
	4.00 p.m.	60	60	60
	10.00 p.m.	65	65	65
10	10.00 a.m.	70	75	70
	10.30 a.m.	60	60	60
	4.00 p.m.	60	60	65
	10.00 p.m.	65	65	65
	10.00 a.m.	80	80	75

1. Doses of I.V. Ismelin in mg./kg./24 hours

2. Blood pressure in mm.Hg.



Text Fig. 1 A multiple graph showing serial blood pressure estimations in the first group of two animals. Estimations were made at 6 hour intervals for a period of 48 hours. The larger figure on the left (in circles) show the animal experimental number. The smaller figures show blood pressure in mm.Hg. This group received smaller doses of Ismelin.



Text Fig. 2 A multiple graph showing serial blood pressure estimations in the second group of three animals. Estimations were made at 6 hour intervals for a period of 48 hours. The larger figures on the left (in circles) show the animal experimental number. The smaller figures show blood pressure in mm.Hg. This group received larger doses of Ismelin.

Experiment 2

The study of total tissue pressure (TTP) in normal testis and in tumour tissue (Brown-Pearce carcinoma) implanted in the contralateral testis. The estimations were carried out in normotensive and hypotensive rabbits.

The objectives of these experiments were (1) to make a quantitative study of the TTP prevailing in normal rabbit testes and in intratesticular tumour in normotensive rabbits, (2) to study the effects of induced hypotension on these values and (3) to find whether the needle-pressure measurement is a satisfactory and reliable method for estimating the "pressure" prevailing in a neoplastic tissue as compared with that in a normal tissue.

Materials and Methods

Healthy rabbits of mixed strain and weighing 2-3 kg. were used. Fresh fragments of Brown-Pearce carcinoma from a donor animal were implanted into the right testicular parenchyma using standard lumbar puncture needles, and the contralateral testis was used as a control. The procedure was performed on ten animals. The blood pressure was recorded from the central artery of the rabbit ear using the Grant capsule.

Estimation of the TTP using the "needle-pressure" technique was carried out. The technique used in this work is similar to that described by Young, Lumsden and Stalker (1950) and Shivas (1955) namely the "needle-pressure" technique. The apparatus (Fig. 5) consists

essentially of two graduated manometers - a vertical water manometer and a horizontal capillary manometer, 2 mm. in internal diameter, with a needle carrier at one end. The two manometers are joined with a rubber tube through a T-tube to a mouth-piece. By gently blowing this mouthpiece a known pressure is applied to both manometers. The capillary manometer is in the same horizontal plane as the tissue under examination. This manometer carries a 21 B.W.G. needle with a blind end, and two small lateral holes bored through its wall proximal to the blind end; it is charged with a 3.8 per cent solution of sodium citrate which is coloured red with safranin. The needle of the apparatus is inserted into the testis or the tumour, and pressure is gradually applied by blowing through the mouthpiece. A displacement of the column of fluid in the vertical manometer occurs, while the fluid column in the capillary manometer remains stationary until the pressure gradually rises and leads to the movement of the capillary meniscus towards the tip of the needle. The pressure required to cause a slow and uniform movement of the capillary meniscus is recorded on the vertical manometer and represents the TTP. Care is taken to ensure that all connections are air-tight and no air is trapped in the capillary manometer.

On the twelfth day after implantation of the tumour, each animal was anaesthetised with nembutal supplemented with ether inhalation. Hypotension was induced by the intravenous injection of Ismelin in a dose of 10 mg/kg/24 hours. Estimations of the TTP were

made in the normal testicular tissue and the tumour tissue implanted and growing in the contralateral testis. These estimations were made before, and approximately thirty minutes after induction of hypotension, using the technique already described.

Results

The results of a typical experiment are illustrated in Text Figure 3. The results of the TTP estimations in all animals under experiment are summarised in Text Figure 4.

The same experiment was repeated on the nineteenth day after implantation, to see the effect of the lapse of time on the TTP. The results are also summarised in Table 3.

Comments

The conclusions from the experiments are:-

1. The TTP of the Brown-Pearce carcinoma implanted and grown in the rabbit testis is much higher than that of the normal testis and increases with the lapse of time.
2. Administration of Ismelin for inducing hypotension in the animal circulation causes a decrease in the TTP which varies in different animals although the TTP of the tumour remains significantly higher than that of the normal (control) testis.
3. The pressure readings observed in the normal and the tumour tissue were above zero. Each reading represents a record of the pressure required to force a small quantity of fluid into the tissues to counteract the forces mutually exerted by the

tissue cells at their interfaces. These "positive" pressure readings were taken as indication of the validity of the needle-pressure method.

4. There was a correlation between the size of the tumour and the tissue pressure; the tissue pressure was greater in larger tumours as indicated in Table 3.

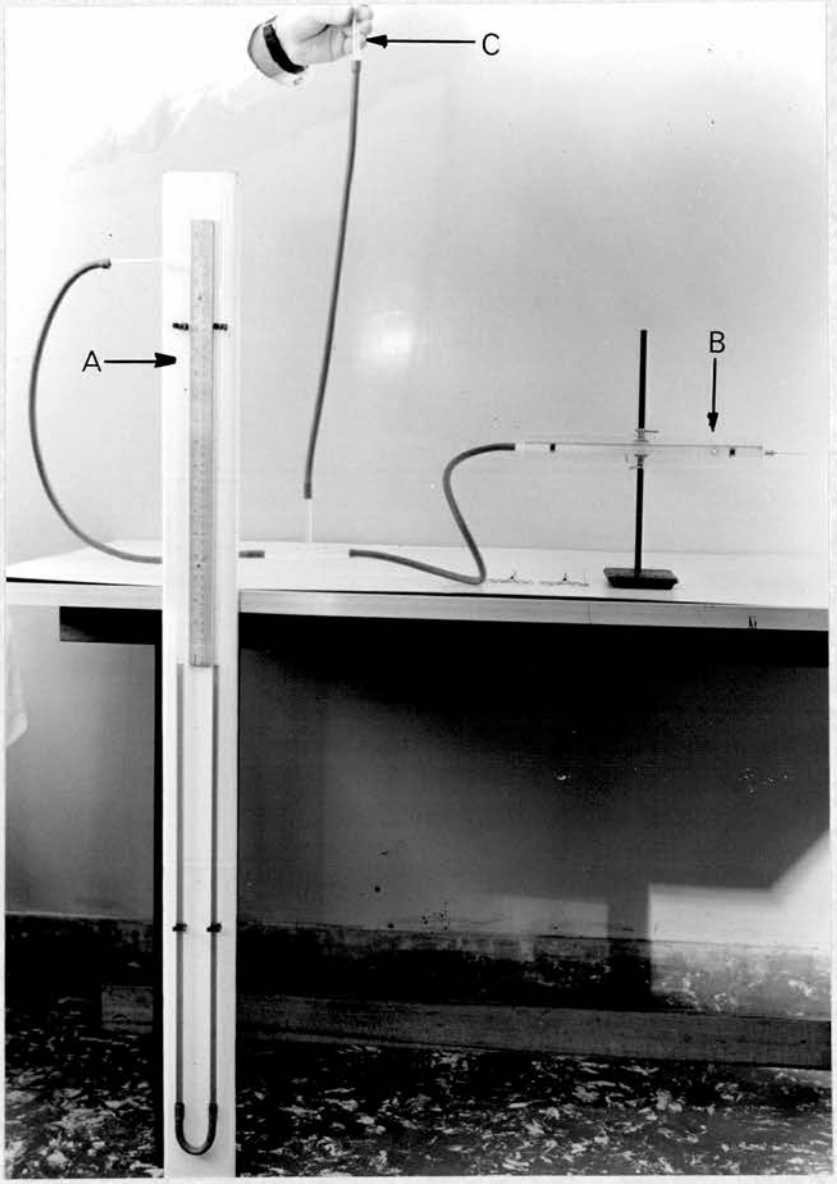
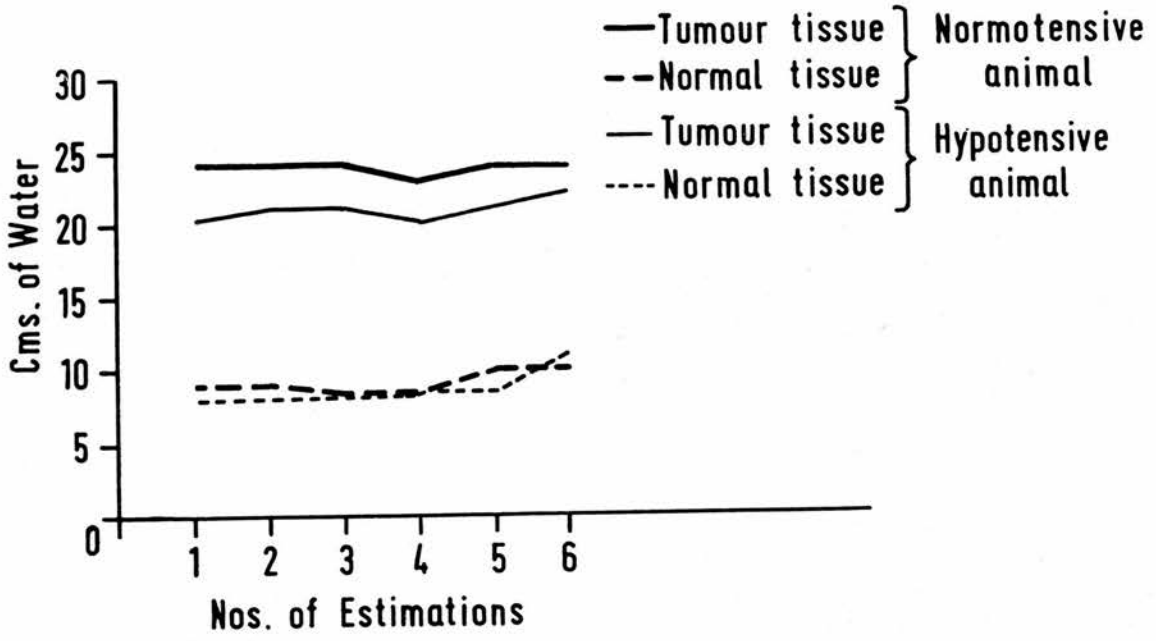
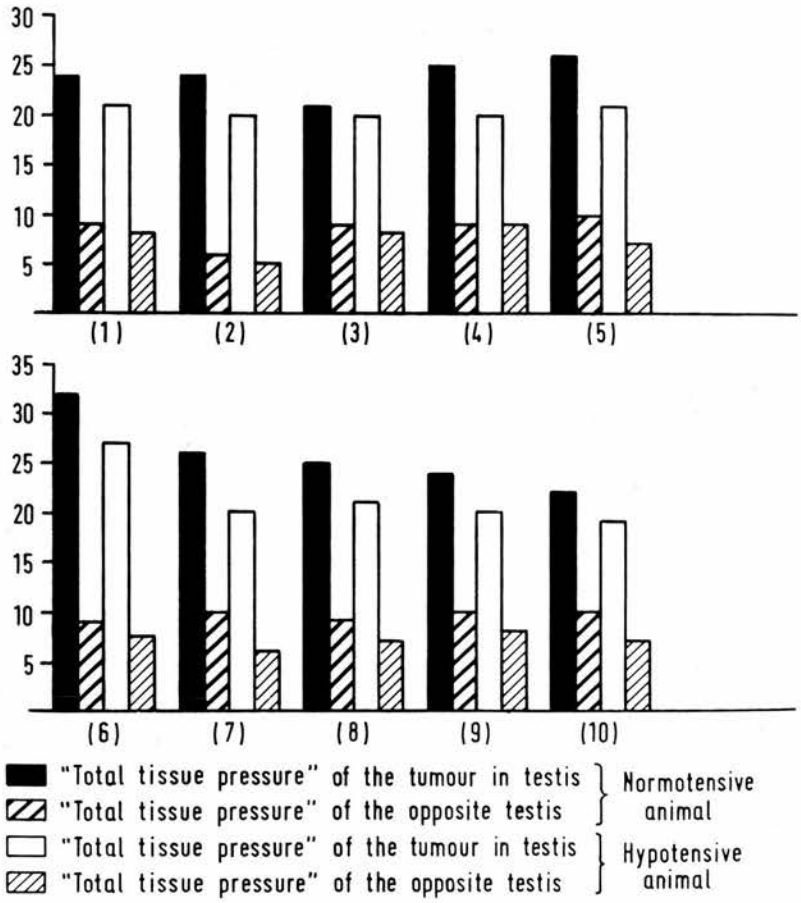


Fig. 5 "Total tissue pressure apparatus".
A. vertical water manometer; B. capillary manometer carrying needle; C. mouthpiece



Text Fig. 3 Graphic recording of the total tissue pressure in rabbit No. 1 before and 30 minutes after administration of intravenous Ismelin, on the 12th day of implantation of the Brown-Pearce carcinoma.



Text Fig. 4 A histogram showing total tissue pressure estimations in 10 rabbits before and 30 minutes after induction of hypotension by intravenous Ismelin, on the 12th day of implantation of the Brown-Pearce carcinoma.

TABLE 3

Total Tissue Pressure of Brown-Pearce Carcinoma Estimated on the 12th and the 19th day of Implantation in Normotensive Animals and 30 minutes after Administration of Ismelin

Animal Nos.	1. T.T.P.T. Normotensive Animal		T.T.P.T. Hypotensive Animal	
	12th day	19th day	12th day	19th day
1	24	28	21	25
2	24	26	20	22
3	21	24	20	21
4	25	30	20	24
5	26	28	21	28
6	32	36	27	32
7	26	28	20	26
8	25	28	21	29
9	24	30	20	30
10	22	24	19	18

1. Total tissue pressure of the tumour.

N.B. There was no change in the total tissue pressure of the normal testes with the lapse of time.

Experiment 3

A study of relationship between hypotension and necrosis in Brown-Pearce carcinoma.

The object of this experiment was to study the effect of hypotension on the occurrence and extent of necrosis in intratesticular Brown-Pearce carcinoma as compared with normotensive tumour-bearing control animals.

Materials and Methods

Fifteen healthy mixed-strain rabbits each weighing 2-3 kg. were used. All were normotensive (Grant capsule estimation). They were prepared in the usual way with intratesticular tumour implants which were large and readily palpable tumours by the sixteenth day. At that stage the animals were divided into two groups. Group A (the experimental group) comprised ten rabbits in which hypotension was induced by Ismelin (10 mg./kg. body weight). Two intravenous injections were given, the second 24 hours after the first, to ensure that the hypotension would be maintained for 48 hours. Blood pressure readings were made every 6 hours and the animals were killed on the eighteenth day. Group B, five rabbits, formed the control group killed on the sixteenth day, at the point when the hypotension began in group A. In both groups the same procedure was followed after they were killed. The testes were removed, the lesions dissected carefully, the gross and microscopic extent of necrosis in the tumours were studied. In all animals post-mortem examinations

were made and representative blocks of tissue taken from brain, liver, kidney, lung, muscle and adrenals.

Results

The tumour had grown satisfactorily in all the rabbits. In Group A, the blood pressure was maintained between 55-75 mm.Hg. over a period of 48 hours. Apart from a few side-effects of Ismelin, such as diarrhoea and tachycardia, the animals looked healthy and no pathological lesions could be seen on either the gross or the microscopic appearance of the tissues examined. In seven animals there was obvious widespread necrosis, the other three showed moderate and slight degrees of necrosis. The controls showed slight degrees of necrosis. The findings are summarised in Tables 4 and 5. The gross and the microscopic appearances are described below.

Gross Observations

In group A most of the tumours had altered colour. The usual greyish colour had changed into yellowish-brown, or greenish-brown and brownish-red as a result of congestion and haemorrhage from the damaged capillaries. Zones of congestion and haemorrhage separated the necrotic tissue from the viable parts of the tumour. The necrotic tissue was not always confined to the centres, a phenomenon which will be referred to later. Some tumours were surrounded by normal testicular parenchyma, while in other places the tumours infiltrated the whole testes (Figs. 6, 7 and 8). In group B the tumours showed little or no necrosis (Figs. 9 and 10). The tumours illustrated in Figs. 7 and 10 appear to be largely extratesticular.

These tumours were not included in the experiments in which the tumour pressure was measured.

Microscopic Observations

Histological examination reveals widespread necrosis, much of it haemorrhagic in type, while many cells not entirely necrotic show marked nuclear pyknosis (Figs. 11 and 12). The viable tumour tissue is not confined to the periphery only and the cords of tumour cells are seen scattered within the necrotic tissue, mainly around intact vessels (Figs. 13 and 14). Viable tumour is seen also to have infiltrated the testicular parenchyma, though in many places the necrotic tumour is surrounded by a rim of compressed testicular tissue with lymphocytes and plasma cells infiltration. Between the viable and the necrotic tissue some tumours have a band of polymorphonuclear leucocytes (Figs. 15, 16, 17 and 18).

Microscopic examination confirmed the slight extent of the necrosis in the control tumour (Figs. 19 and 20).

Comment

There was a considerable variation in the size of the tumours at 16-18 days after implantation as demonstrated in figures 6-10. The experiments demonstrate that hypotension induced and maintained for 48 hours in rabbits bearing intratesticular Brown-Pearce carcinoma is associated with the development of necrosis in these tumours, and that necrosis is significantly greater than that in the control tumours. It has to be noted that this is a conditioned experiment

and is influenced considerably by the age and the size of the implanted tumour.

The difference in age of the control and experimental animals' tumours, therefore, could have some effect on the results, since necrosis increases with age of the tumours, but necrosis was significantly greater than in the controls. This difference was much greater than might be anticipated from a tumour age difference of two days. As emphasised above, the experiment was timed to precede the massive necrosis which occurs spontaneously in these lesions, usually after the twenty-first day of implantation. Even so, there was a considerable variation in the extent of the necrosis which clearly bore a direct relationship to the size of the tumour at the time of the hypotension.

TABLE 4

Summary of the Experiments

Animal Experimental Number	Tumour Transplantation	Remarks	Necrosis
1	Both testes	Experimental	+++
2	"	"	++
3	"	"	+++
4	"	"	+
5	"	"	+++
6	"	"	+++
7	"	"	++
8	"	"	+++
9	"	"	+++
10	"	"	+++
11	"	Control	+
12	"	"	+
13	"	"	+
14	"	"	+
15	"	"	+

\+ slight

++ moderate

+++ severe

TABLE 5

The Effect of Ismelin on Peripheral Blood Pressure and Tumour Behaviour

Animal Exp. No.	Animal[1] Blood Pressure	Dose[2] Ismelin I.V.	Blood Pressure Recording-Time after Ismelin Injection								Remarks
			10.00 a.m.	10.30 a.m.	4.00 p.m.	10.00 p.m.	10.00[3] a.m.	4.00 p.m.	10.00 p.m.	10.00 a.m.	
1	110	10	115	65	60	60	70	60	55	65	Widespread necrosis
2	120	10	120	65	60	70	75	65	65	70	Moderate necrosis
3	110	10	115	70	65	70	75	65	65	70	Widespread necrosis
4	120	10	120	65	60	55	65	60	60	65	Slight necrosis
5	110	10	110	55	55	60	65	55	60	65	Widespread necrosis
6	120	10	120	60	60	65	65	60	60	65	Widespread necrosis
7	120	10	125	70	60	65	70	60	65	70	Moderate necrosis
8	120	10	120	65	60	65	70	60	60	65	Widespread necrosis
9	120	10	125	70	65	65	70	65	65	65	Widespread necrosis
10	120	10	120	60	60	65	70	55	60	65	Widespread necrosis
11	120	-									Slight necrosis
12	110	-									Slight necrosis
13	120	-									Slight necrosis
14	110	-									Slight necrosis
15	120	-									Slight necrosis

1. Blood pressure is measured in millimetres of mercury.
2. Ismelin doses are in milligrammes per kilogrammes body weight.
3. Another dose of Ismelin is given.

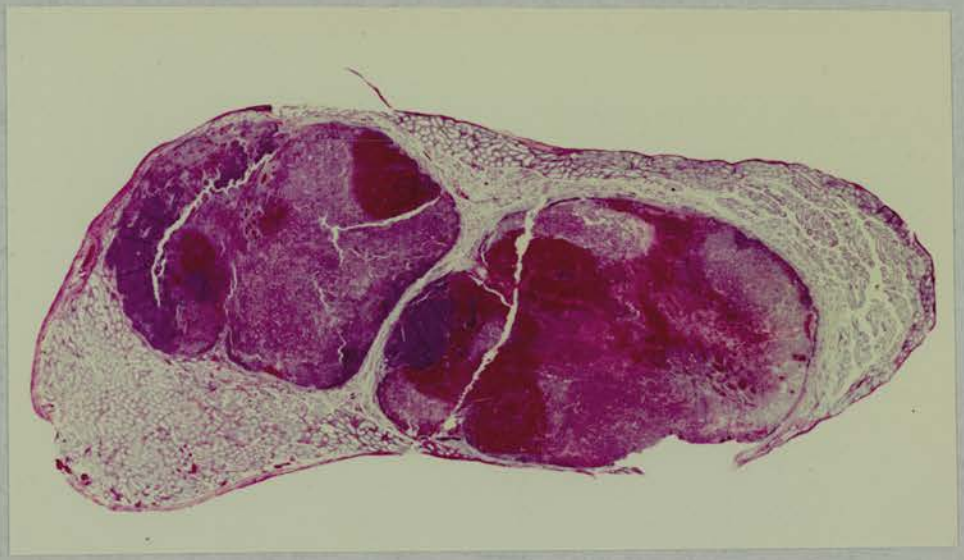


Fig. 6 A complete transection of rabbit testis showing two nodules of Brown-Pearce carcinoma, 18 days after implantation. Systemic hypotension was induced for 48 hours and widespread haemorrhagic necrosis has occurred. Note that only the areas stained dark blue remain viable.

H. and E. x 5.

Fig. 7 Transection of rabbit testis showing Brown-Pearce carcinoma nodule, 18 days after implantation. Systemic hypotension was induced for 48 hours and widespread haemorrhagic necrosis has occurred. Note the tumour was growing outside the testicular parenchyma apparently along the lines of least resistance. Thus, Brown-Pearce carcinoma can grow outside the testicular parenchyma but behaves similarly to that growing within it. Compare with Fig. 10.

H. and E. x 4

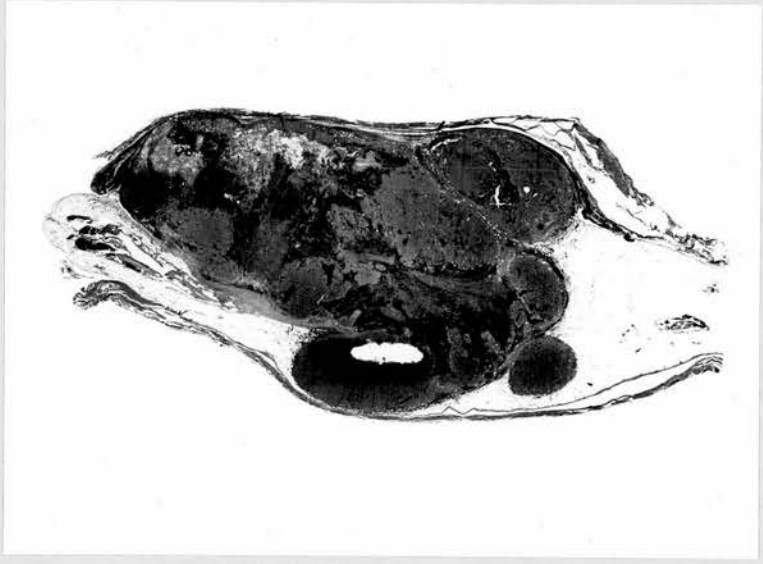


Fig. 8 Transection of rabbit testis showing Brown-Pearce carcinoma nodule, 18 days after implantation. Systemic hypotension was induced for 48 hours and widespread haemorrhagic necrosis has occurred. The viable tumour is mainly around the periphery.

H. and E. x 4

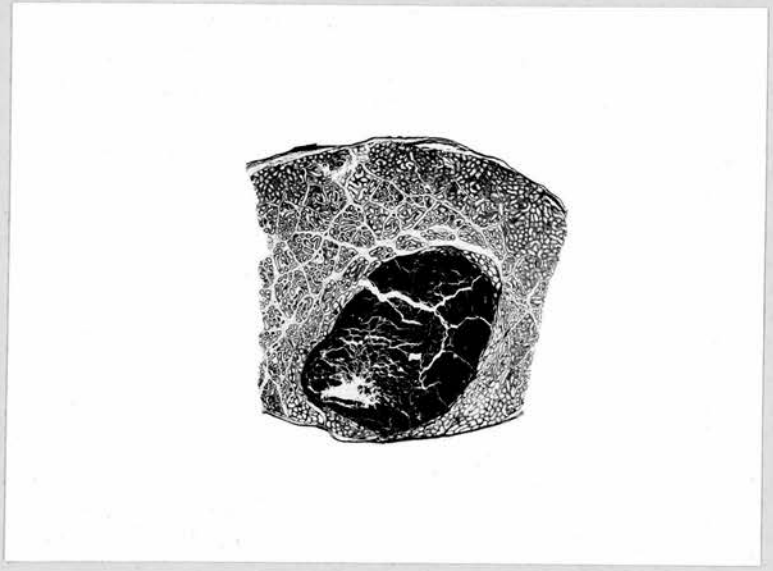


Fig. 9 Transection of rabbit testis showing Brown-Pearce carcinoma nodule, 16 days after implantation in a control animal. The tumour is viable and necrosis is not seen on the gross appearance. Note the absence of the pale, necrotic areas, seen in Fig. 8.

H. and E. x 4

Fig. 10 Transection of rabbit testis showing Brown-Pearce carcinoma nodule, 16 days after implantation in a control animal. Note that only a small focus of necrosis is present, near the upper margin of the tumour. A separate smaller nodule of tumour, entirely viable, is also present (lower right).

H. and E. x 5

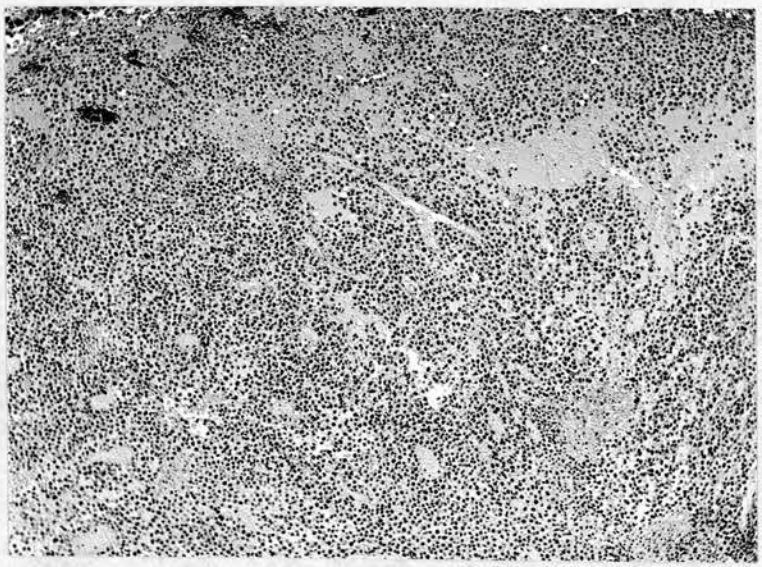


Fig. 11 Histology of the tumour shown in Fig. 6 with widespread necrosis. Note that necrosis is mostly haemorrhagic; both intact and damaged vessels are seen throughout.

H. and E. x 60

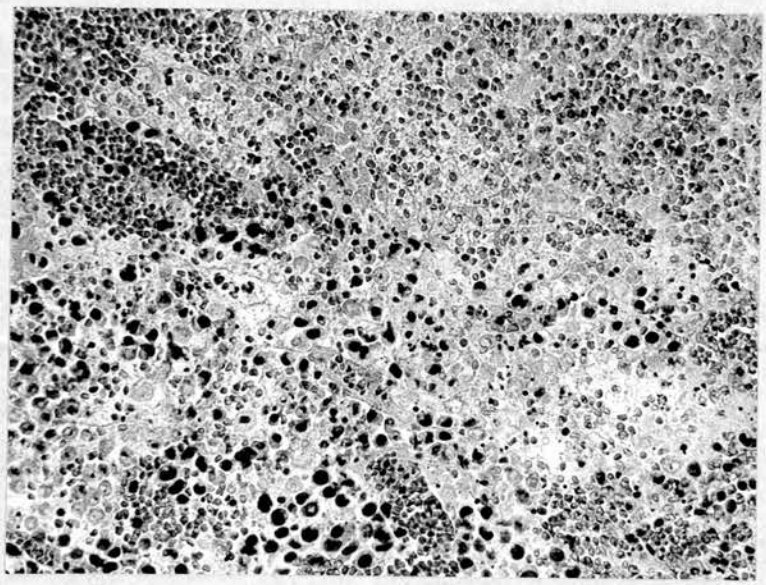


Fig. 12 Detail of Fig. 11 showing haemorrhagic necrosis and many cells with pyknotic nuclei. These are mainly around intact vessels (bottom). Note dilated and damaged vessels within the necrotic tissue.

H. and E. x 275

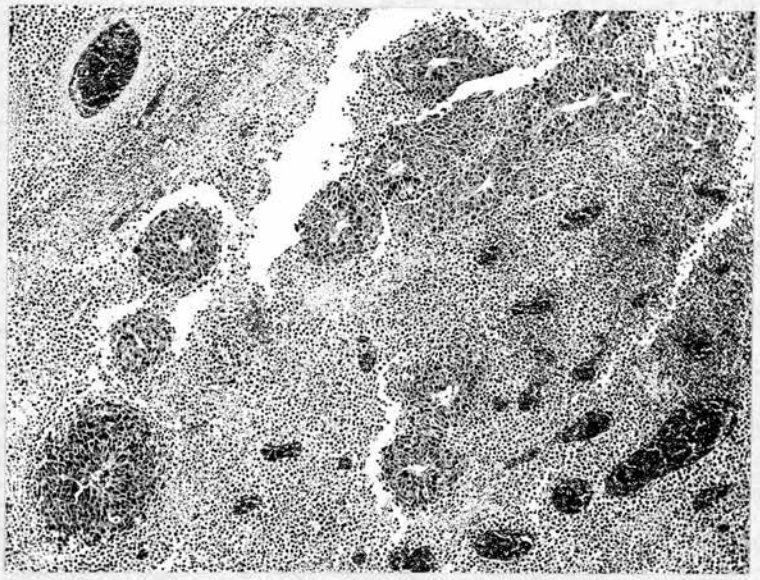


Fig. 13 Section of Brown-Pearce carcinoma in rabbit testis showing viable tumour cords within large area of necrosis. Note many dilated vessels within the necrotic tumour.

H. and E. x 60

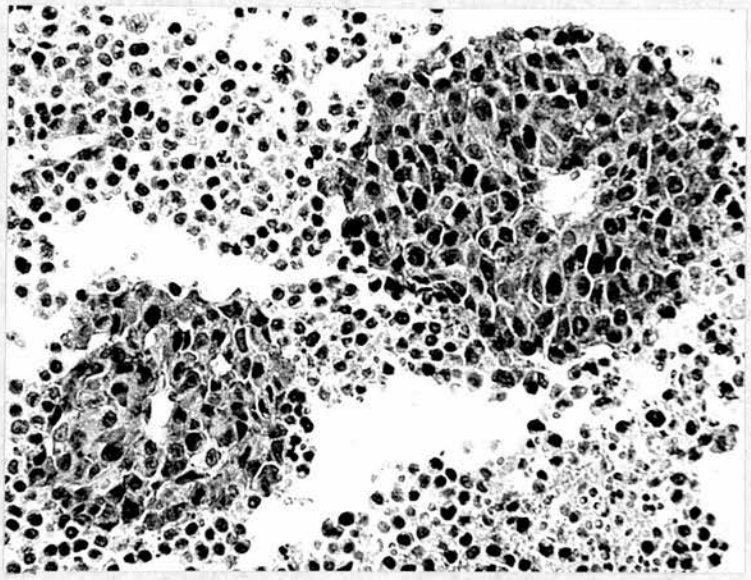


Fig. 14 Detail of Fig. 13 showing two viable tumour cords within a necrotic tissue. Note each cord is nourished by an intact vessel running along its axis.

H. and E. x 275

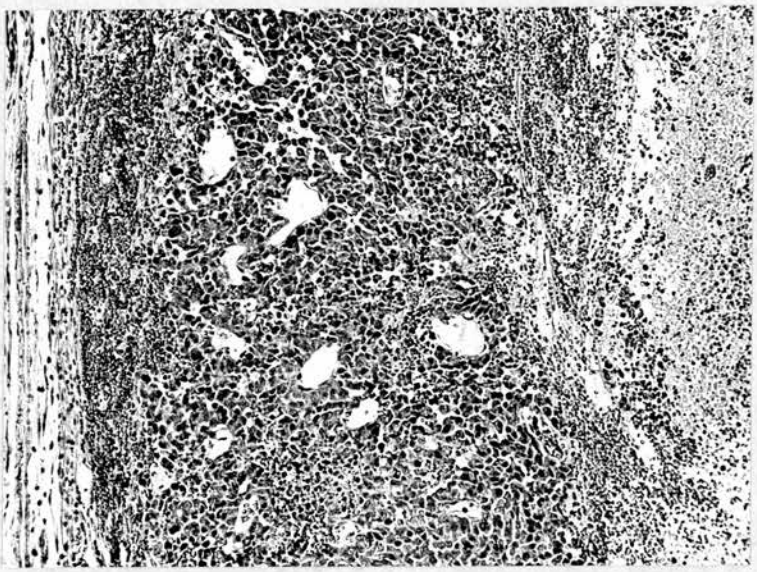
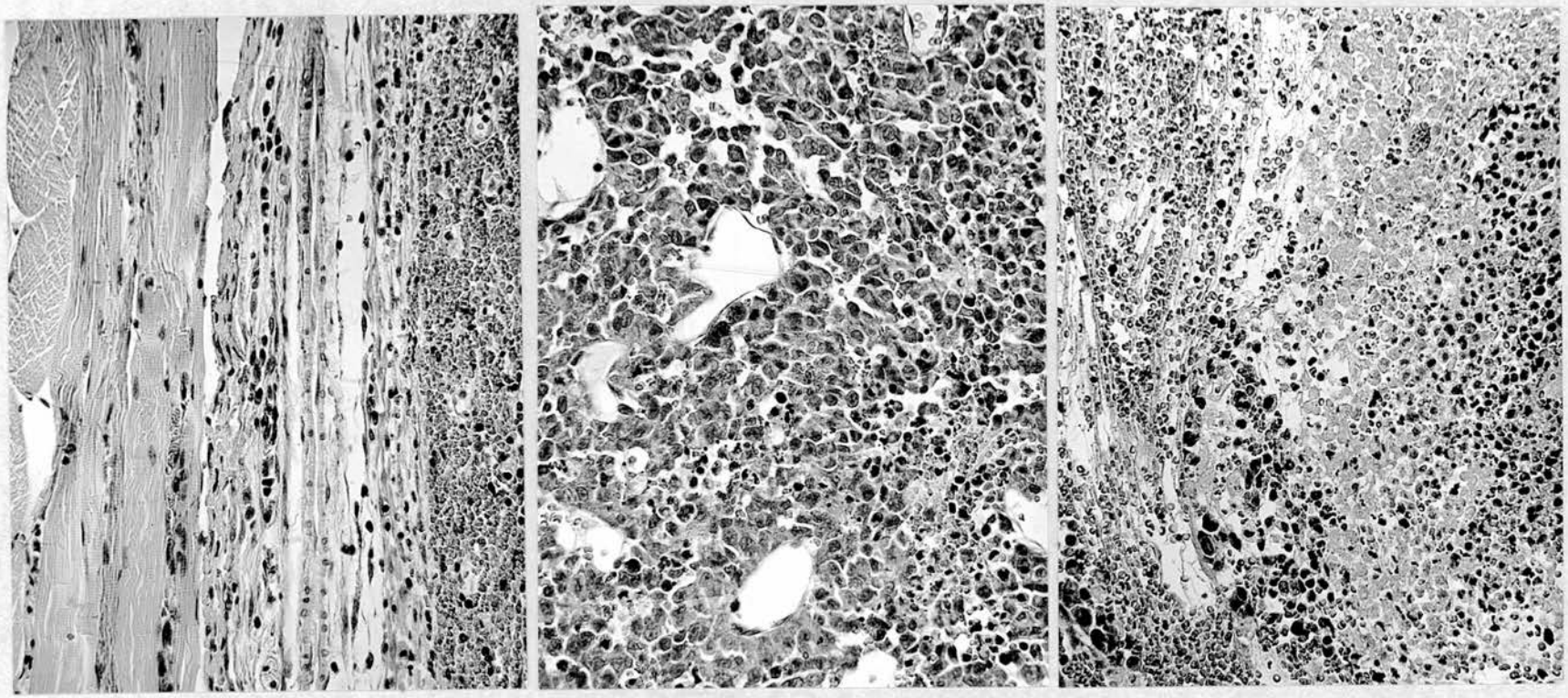


Fig. 15 Brown-Pearce carcinoma in rabbit testis showing highly vascular tumour with many intact small vessels (centre). Note the viable tumour is demarcated from the surrounding testicular tissue (left), and the necrotic tumour (right) is mostly haemorrhagic. Compare the vessels in this figure with those in Fig. 13 and see Fig. 16 for the detail.

H. and E. x 110.



A. B. C.

Fig. 16 Detail of Fig. 15

A. The tissue surrounding the tumour illustrated in Fig. 15, showing muscle fibres (left) and compressed testicular tissue with chronic inflamatory infiltration (middle) surrounding a rim of necrotic tumour (right).
B. Viable tumour. Note high vascularity and minimal necrosis.
C. Necrotic tumour with many cells showing nuclear pyknosis. Note necrosis is mostly haemorrhagic in type.

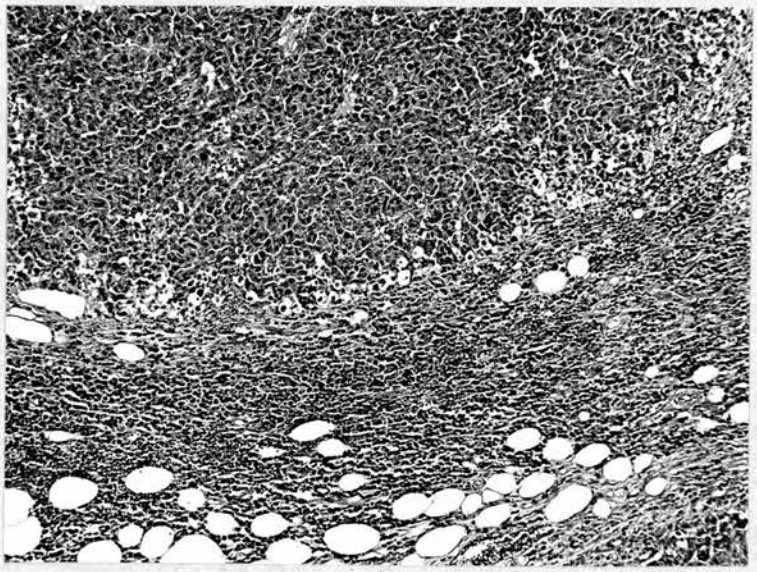


Fig. 17 Brown-Pearce carcinoma with chronic inflamatory infiltration surrounding the tumour.

H. and E. x 90.

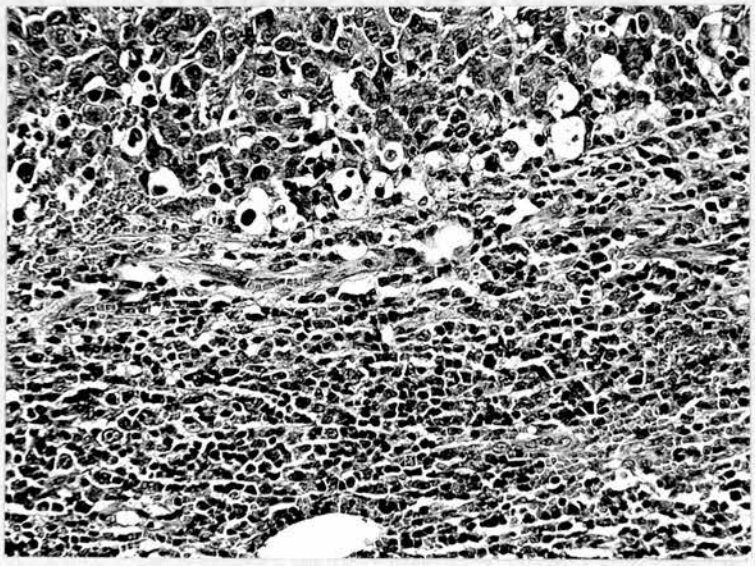


Fig. 18 Detail of Fig. 17. Note that many of the cellular infiltrates around the tumour are lymphocytes.

H. and E. x 250.

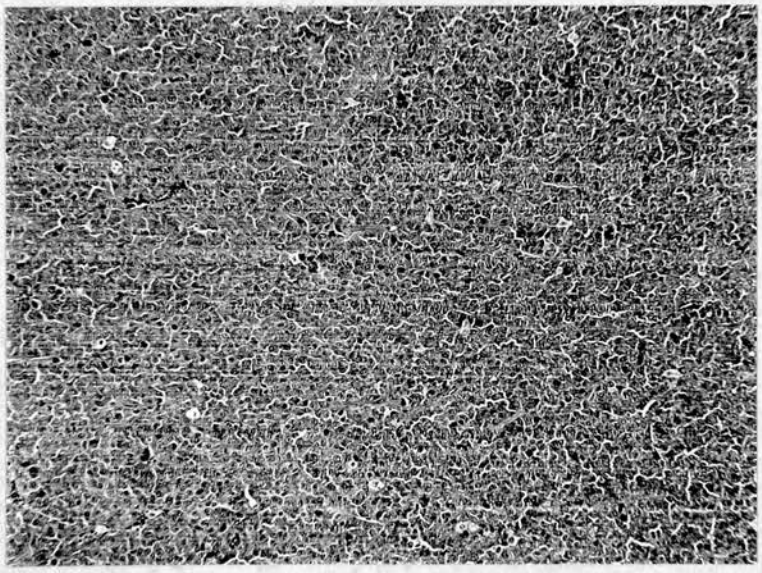


Fig. 19 Brown-Pearce carcinoma growing in rabbit testis. Note minimal necrosis and absence of "architecture" in the tumour. Compare with Fig. 11.

H. and E. x 90

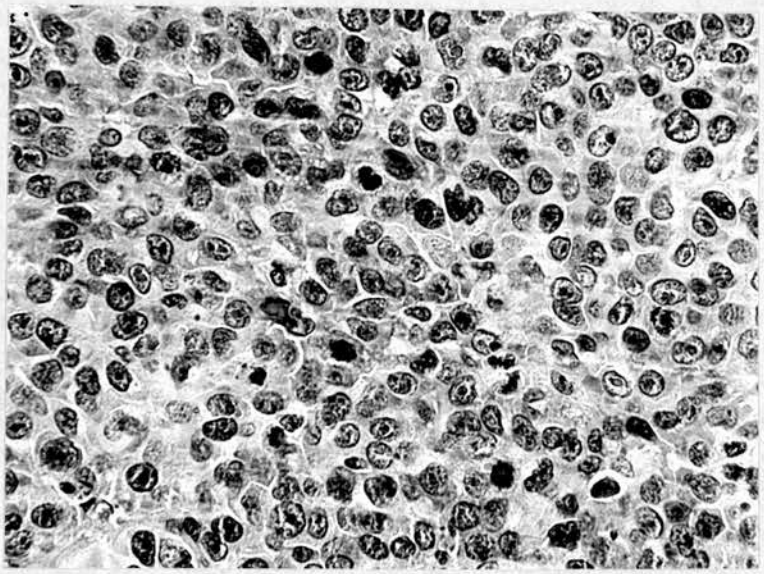


Fig. 20 Detail of Fig. 19. Note that the tumour cells are closely packed and many show mitotic figures.

H. and E. x 425.

CHAPTER 3

Histological Study of Small Blood Vessels in Brown-Pearce Carcinoma

In the previous experiments evidence was presented that necrosis in Brown-Pearce carcinoma grown intratesticularly was closely related to hypotension maintained for many hours in the tumour-bearing animals. The present chapter deals with the vascular morphology and the relation of vascular supply to necrosis in this tumour.

1. Demonstration of small vessels histologically in Brown-Pearce carcinoma

The different techniques which have been used by other workers to investigate the tumour vasculature are summarised in Table 6. In the present investigation one problem has been to demonstrate small blood vessels in the necrotic parts of the tumour and after several attempts it was found that a method incorporating luxol-fast blue was satisfactory. The technique was developed originally by Klüver and Barrera (1953) to demonstrate myelin in the central nervous system. Subsequently, Margolis and Pickett (1956) used this stain in combination with the periodic acid-Schiff method plus haematoxylin to produce maximum contrast in animal tissues. They found that among other tissues which tend to bind luxol-fast blue are muscles, connective tissue and red blood cells. Tannock and Steel (1969) used this method of staining to estimate the distance of a tumour cell from its nearest capillary in experimental mammary adenocarcinoma in rats in order to correlate this distance with the oxygen diffusion length.

In the present study a combination of periodic acid-Schiff and luxol-fast blue were used with haematoxylin, to give a good contrast between the vascular endothelium and the erythrocytes. The following staining procedure was used. It is slightly different from that used by Margolis and Pickett (1956):-

After fixation in 10% formalin and paraffin embedding, 4μ sections were cut, dewaxed and placed in 95% alcohol.

1. Slides were placed in luxol-fast blue solution (0.1% L.F.B. in 95% alcohol) at 60°C overnight, rinsed in 95% alcohol and distilled water, and differentiated in 0.05% lithium carbonate solution and 70% alcohol for a few seconds, and then rinsed again in distilled water.
2. Slides were placed in 0.5% periodic acid-Schiff solution for 5 minutes, then washed in distilled water, immersed in Schiff solution for 20 minutes, and then the slides were given three changes of sulphurous acid solution for 2 minutes each, then washed in tap water for 5 minutes before staining in haemalum.
3. After alcohol dehydration and clearance in xylene, the slides were mounted in Canada balsam.

In sections stained by this method, purplish-blue or bluish stained erythrocytes were visible, giving a good contrast with red-stained vascular endothelium.

Results

By this method vessels with intact walls were seen in viable and in necrotic regions of the tumour, although the latter also

contained damaged vessels. It was of interest to note that vessels in the necrotic regions were dilated with high concentrations of red cells whereas this was not the case in the viable tumour (Figs. 21, 22, 23, 24 and 25). Thus the salient points in these observations are:-

1. Viable tumour has intact vessels.
2. Necrotic tumour has injured as well as intact vessels.
3. Vessels in the necrotic tumour are dilated with high concentrations of red cells in each vessel.
4. Small vessels appear to enter the tumour from the periphery; and necrosis is not always confined to the central part but is scattered throughout the tumour.

TABLE 6

Published Work on Microcirculation of Tumours
Methods of Study and Review of Literature

Method of Study	Author and Year
Histology	Lindgren (1945) Coman and Sheldon (1946) Thomlinson and Gray (1955) Scheid (1961)
Dye injection and histology	Goldman (1907) Lewis (1927) Waters and Green (1959) Goldacre and Sylvén (1962) Lien and Ackerman (1970)
Cast injection of blood vessels	Gullino and Grantham (1962) Shivas and Gillespie (1969)
Angiography	Hasegawa (1934) Shinkawa (1939) Braithwaite (1958)
Microangiography	Lagergren et al. (1958) Lagergren et al. (1960) Chang and Trembly (1961) Rubin et al. (1964) Rubin and Casarett (1966)
Transparent chamber	Ide et al. (1939) Algire (1943) Algire and Chalkly (1945) Algire and Legallais (1951) Williams (1951) Goodall et al.(1964), (1965) Warren (1967) Witte and Goldenberg (1967) Sanders (1971)

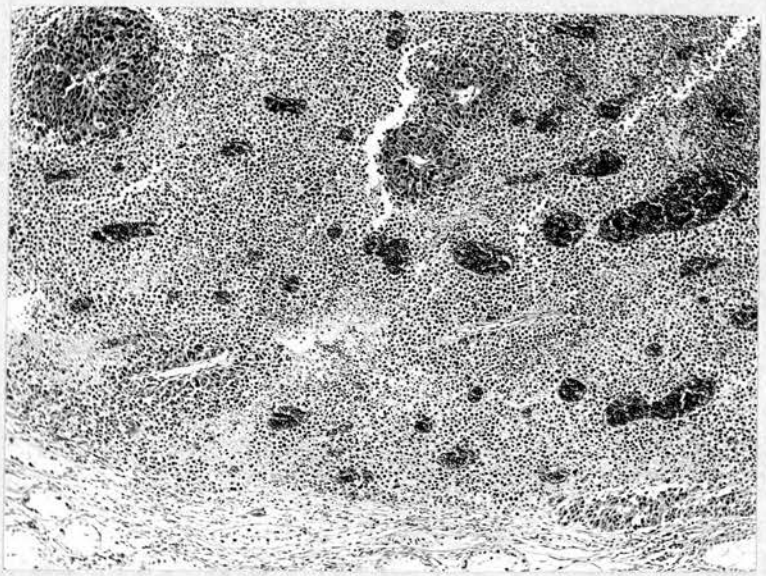


Fig. 21 Section of Brown-Pearce carcinoma in rabbit testis showing widespread haemorrhagic necrosis and a few viable tumour cords (top). Note the many dilated and congested vessels within the necrotic tissue. Each tumour cord is supplied by a smaller vessel running along its axis. The necrotic tumour is surrounded by the compressed testicular parenchyma.

H. and E. x 60.

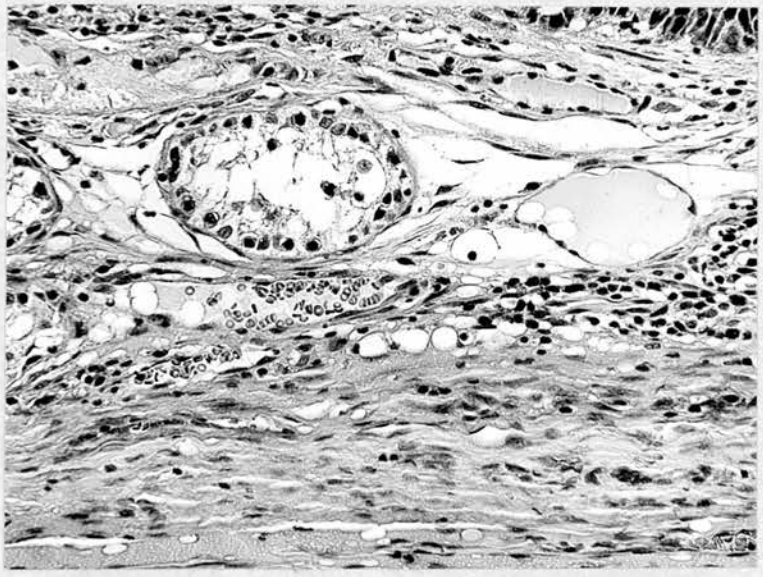
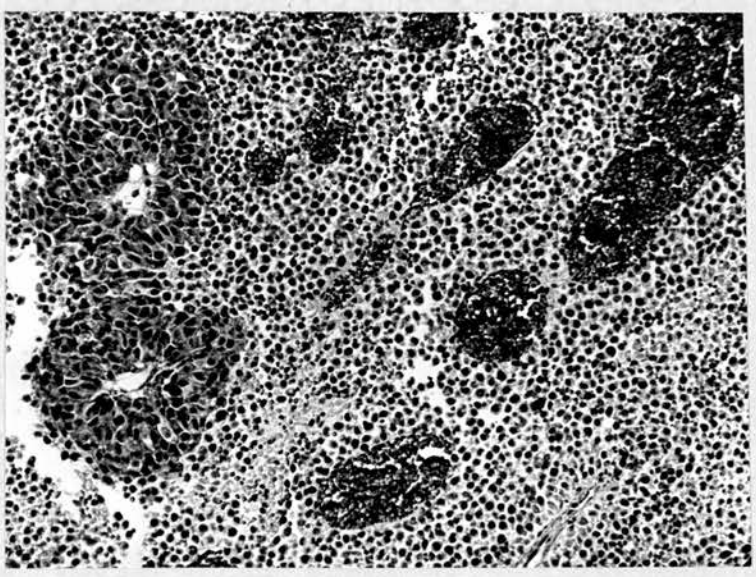


Fig. 22 Detail of Figure 21.

A. The tissue surrounding the tumour shown in Fig. 21 showing chronic inflamatory infiltration. Note many vessels and testicular tubules.

H. and E. x 250



B. Two viable tumour cords (left) and many dilated vessels packed with red cells within the necrotic tumour. Compare these vessels with those seen in the centre of the tumour cord and those shown in Fib. 23.

H. and E. x 160

Fig. 23 Dilated vessels within necrotic Brown-Pearce carcinoma in the rabbit testis. Note many cells are not completely necrotic, mainly seen around blood vessels. These vessels may well be physically and functionally abnormal.

H. and E. x 110

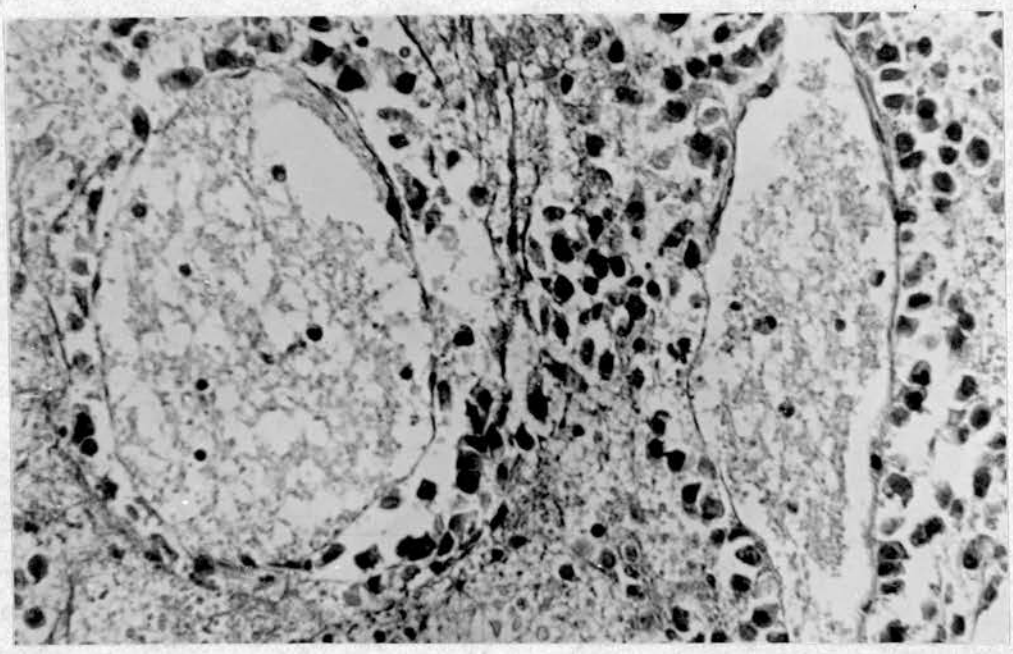


Fig. 24 Dilated vessels in necrotic Brown-Pearce carcinoma in the rabbit testis. The basement membrane is well demonstrated with this stain.

luxol-fast blue x 550

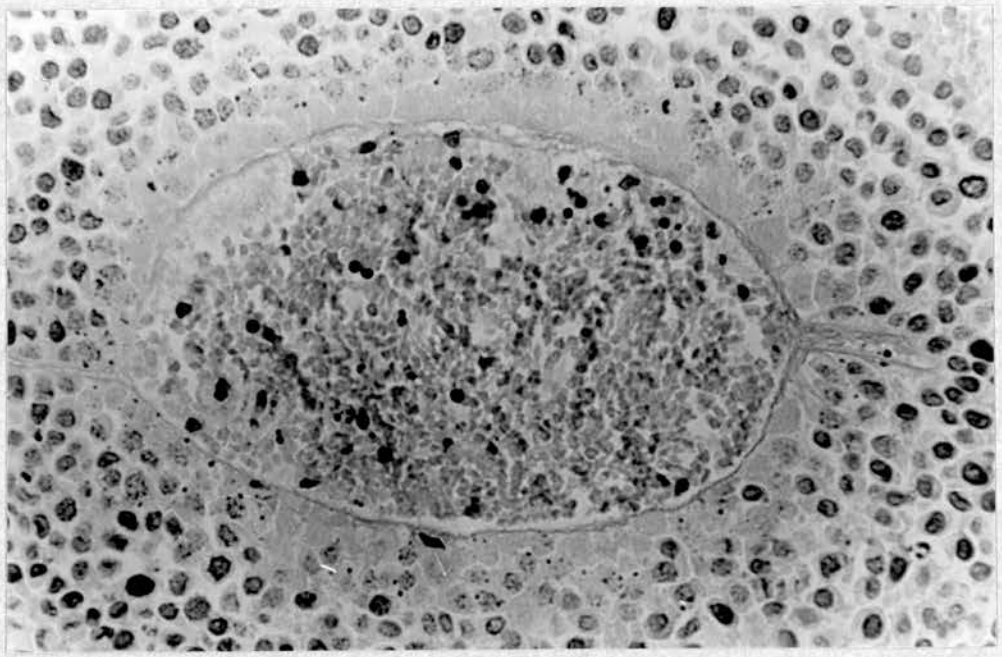


Fig. 25 High power view showing vessel in necrotic Brown-Pearce carcinoma. Afferent and efferent vessels are seen in continuity with a greatly dilated, sinusoidal channel.

luxol-fast blue x 550

2. The physical state of the blood in the vessels of Brown-Pearce carcinoma

Compression of the intrinsic tumour vessels under the relatively high tissue pressure of the surrounding tumour can result in slower rate of circulation and even vascular stasis. Indirect evidence of stasis can be obtained by the presence of fibrin in the lumina of vessels, and for this purpose the picro-Mallory method is used. The method was first used by Mallory (1938) as a special stain for collagen fibres. Lendrum et al. (1962) described a modification of the method and showed its application to demonstrate fibrin and to study its relation to other tissues.

The following staining procedure was used, which is only slightly different from that described by Lendrum et al.

Tumour slices 1.0 cm. thick were cut and fixed in formal saline for about 5 hours, then transferred to 5% aqueous mercuric chloride. The preliminary fixation helps to preserve the erythrocytes. The tissue was processed and sections dewaxed in xylene and rinsed with absolute ethanol and then taken to water. After treatment with iodine and hypo and washing in water, they were put in the oxazine and haemalum sequence to stain the nuclei, then rinsed again in tap water. Sections were treated with yellow mordant for 5 minutes and washed in tap water for 1 minute, then stained with fuchsin for 5 minutes and rinsed in tap water, differentiated for 15 seconds and rinsed again in tap water. Slides were mordanted with phosphotungstic acid for 5 minutes and rinsed in tap water, then stained with soluble

blue for 2 minutes, rinsed in tap water and dehydrated, then cleared and mounted in Canada balsam.

N.B. The mordant deviates the fuchsin into fibrin and ideally keeps it out of the erythrocytes.

Results

Vessels containing static blood showed fibrin deposition in the lumen in the form of a network or a solid mass. The fibrin stained red and gave good contrast with yellow-stained erythrocytes, as demonstrated in Figs. 26 and 27.

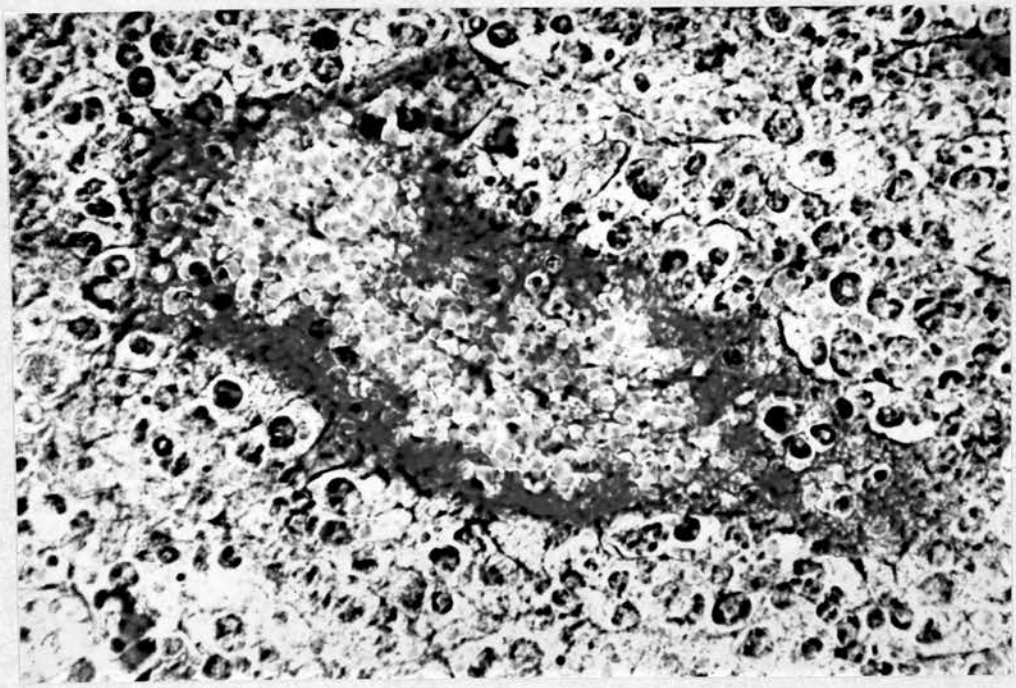


Fig. 26 Section of necrotic Brown-Pearce carcinoma in the rabbit testis. Note the dilated vessel and fibrin deposition (red) in the lumen. Compare with Fig. 27.

picro-Mallory x 550

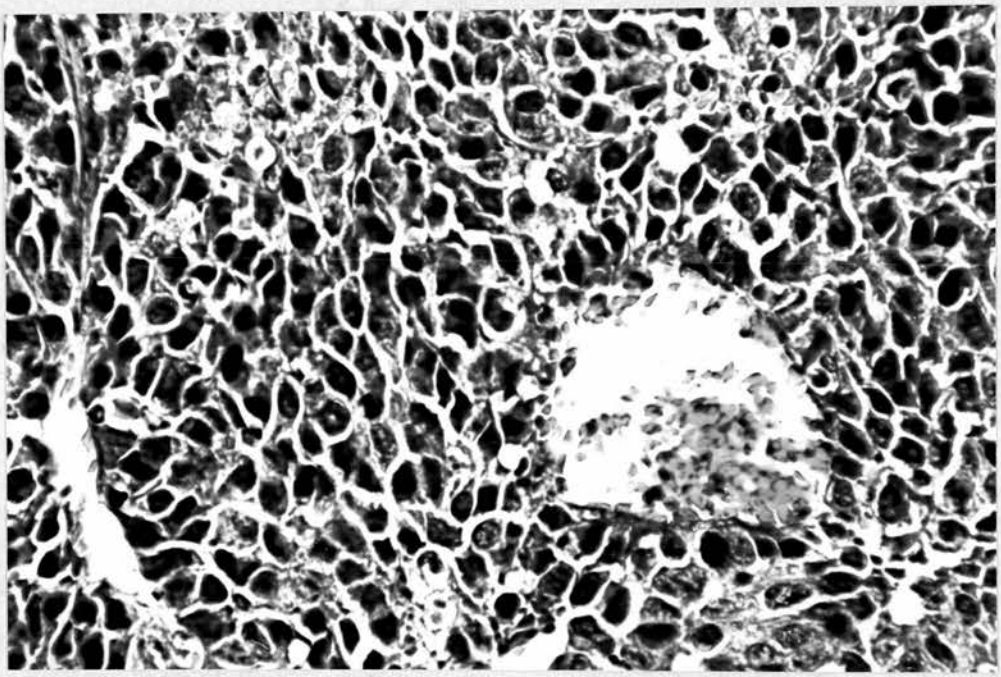


Fig. 27 Section of a viable Brown-Pearce carcinoma in the rabbit testis. Note the absence of fibrin deposition in central vessel.

picro-Mallory x 550

CHAPTER 4

General Discussion and Conclusions

1. The object of the work

The primary object of this part of the work was the study of the effect of hypotension on the behaviour of Brown-Pearce carcinoma grown experimentally in rabbit testis, especially with respect to necrosis, and as a corollary to consider the possible significance of these findings in the treatment of malignancy.

The discussion may conveniently be considered in terms of tumour vasculature, tissue pressure and necrosis in tumours.

2. The vasculature of Brown-Pearce carcinoma

This tumour is widely used in experimental studies and its behaviour is well-documented, especially with respect to its tendency to spontaneous massive necrosis after the twenty-first day in the testicular site. Its rapidity of growth and type of vasculature bear a relationship to such behaviour.

In general it may be said that blood vessels are better developed in slow-growing tumours than in those which grow rapidly; thus, in the former, differentiation into arterioles and venules nearly always occurs, whereas in the latter, the circulation tends to be sinusoidal. This important finding is referred to by many workers including Lewis (1927) and Algire *et al.* (1947) who stated that the more rapidly-growing and more purely epithelial tumours develop a largely sinusoidal type of circulation with no afferent or efferent

vessels such as are found in slowly-growing less cellular tumours.

The Brown-Pearce carcinoma was early recognised to have individual characteristics in morphology and in its sinusoidal type of vasculature. Shivas and Gillespie (1969) described the vascular morphology of the tumour when growing in rabbit liver utilising an injection technique with neoprene latex. They found that the vasculature displayed a characteristic appearance of twisted, strap-like vessels much larger than the liver sinusoids. Their injection method failed to reveal smaller vessels. They also gave evidence that the tumour is nourished almost exclusively by the hepatic artery; in that respect behaving like metastatic carcinoma in human liver. The presence of enlarged capillaries with the appearance of sinusoids with little tendency for differentiation into arterioles or venules is also a characteristic of the tumour growing in rabbit testis as demonstrated in the present study. Similar vascular morphology was seen in the Brown-Pearce carcinoma growing into the rabbit ear (Ide *et al*. 1939), and to some extent in the rabbit brain (Shivas, 1959). It may thus be concluded that the tumour vasculature is almost identical in pattern irrespective of the site of the growth.

The constancy of the blood vessel structure was an important factor in planning the experiments which form the basis of this thesis, namely that hypotension was a factor directly concerned with necrosis in Brown-Pearce carcinoma.

The general plan was designed to investigate the effect of

hypotension induced experimentally on the extent of necrosis in the tumour. The hypotensive experiments were carefully timed to exclude the complication of spontaneous necrosis which usually becomes a feature after the third week.

The mechanism by which the induced hypotension led to haemorrhage and necrosis in the tumours is difficult to explain. This is because of the difficulty of obtaining information about the effect of Ismelin on the amount of blood flowing through the tumours, and on the pressure within the small blood vessels within the tumour. However, compression of the tumour vessels under the raised tissue pressure noted in my experiments seems a possible explanation. This hypothesis received support from the experimental findings of several workers, as Lewis (1927), Williams (1951), Young et al. (1950) and Sanders (1971).

Vascular dilatation and blood stasis of the tumour vessels was shown histologically in the present study. However, neither my experiments nor those recorded in the literature shed any light on the question of whether tumour vessels composed solely of endothelium respond to the hypotensive drug in the same way as muscle-invested small vessels in normal tissue.

3. The meaning of tissue pressure and its measurements: the importance of relative rather than absolute pressures

Quantitative studies on the physical properties of tumours and their relation with the behaviour of that tumour have been few until recently. Young, Lumsden and Stalker (1950) demonstrated that the "tissue pressure" of Brown-Pearce carcinoma implanted in the

rabbit testis is consistently much higher than that of the normal testis and it increases with the lapse of time. The "tissue pressure" measured by Young and his co-workers is the pressure required to introduce a bland fluid into the tissue through a hollow needle; the method used in the present research. Shivas (1955), using the same manometric method, showed that this was also true in Brown-Pearce carcinoma grown inside the brain, even in the presence of raised intracranial pressure. He defined the tissue pressure as the minimum hydrostatic pressure which must be exerted by a thin-walled blood vessel if it is to remain patent. It is thus in effect also the pressure exerted from all directions which tends to obliterate such collapsible structures as the peripheral parts of the tubules and vessels, or thin-walled capillaries. So it may be referred to appropriately as the "total tissue pressure" (TTP).

In recent years the validity of the manometric method for estimating the tissue pressure has been questioned (Guyton, 1963, Guyton and Coleman, 1968 and Ladegaard-Pederson, 1970).

Guyton introduced a method based on pressure measurements in implanted perforated capsules left in situ 4-5 weeks before taking readings. He found that the pressure inside the capsules implanted in the subcutaneous tissue of dogs was "normally negative", this he called the "interstitial fluid pressure". He argued that as the needles used for insertion into the tissues are several hundred times larger than the spaces where the pressure should be recorded, the results are bound to be invalid. In point of fact, however, in these

experiments, what was estimated by the needle method was not the interstitial fluid pressure alone, but the sum of all pressures or forces exerted in all directions by the different tissue constituents, including the pressure caused by fluid in the tissue spaces (interstitial fluid pressure) and the pressure caused by the solid or semi-solid elements, which can be named the solid tissue pressure. Further, encapsulated organs, such as the testis, kidney and eye, or tissues within rigid cavities, as for instance the brain within the skull, certainly have different fluid dynamics from those in the loose subcutaneous tissue from which Guyton and others obtained their results. Moreover, Young and his co-workers (1950) who made a large number of tissue pressure readings, have formed the opinion that the pressure is a particular physical characteristic of the tissue under examination; they believe it to be the most important parameter which opposes the extravasation of blood from a torn or ruptured vessel into the surrounding tissue.

It must be emphasised that the relative pressures rather than the absolute values are the influential factors in my experiments. The significance of this point has been made time and again by Young and Griffith (1950) who have supplied experimental proof that an intraluminal pressure relatively higher than the extraluminal pressure will prevent the entrance of foreign particles (e.g. tumour cells) into these vessels. Accordingly, the effect of hypotension in the experiments which form the basis of this part of the thesis must be interpreted in the context of relative rather than absolute pressures.

More evidence has been presented from different studies that the interstitial fluid of a tumour is physically and biochemically very different from that of normal tissue (Burgess and Sylvén, 1962 and Gullino et al. 1964) and that the interstitial fluid pressure of normal tissues is also above zero (Kjellmer, 1964; Wiederhielm, 1968 and McDonald, 1968). Burgess and Sylvén made a microanalysis of different constituents of interstitial fluid of solid tumours in mice and of the interstitial fluid of normal mice, and found that the composition of the interstitial fluid of solid tumours differs from that of normal tissue. The rise in molarity of tumour tissue, due to the addition of lactate and protein, tends to attract water to the tumour, while the normal outflow and exchanges are probably somewhat retarded. At the same time, the widespread vascular channels, increased permeability and lack of capillary bed within the tumour are factors which contribute to fluid accumulation and retardation of flow due to reduction of the capillary driving force.

The work of Gullino et al., in sampling different types of experimental rat tumours is also of interest in this respect. They used a special draining chamber device to measure the pressure of neoplastic and normal tissue in rats. They found that the tension of normal and neoplastic tissue is positive, and was sufficient to produce a continuous flow from the sampling device. They were able to measure the draining pressure with a mercury manometer and found that the

pressure required to block the draining of interstitial fluid from the diffusion chamber placed in the normal subcutaneous tissue varied from 7-9 mm. of mercury. However, when the chamber was in the tumour, the pressure varied between 8-16 mm. of mercury. They came to the same conclusion that some tumours have a higher tension (pressure) than that of a subcutaneous tissue.

As regards the "tissue pressure" in normal tissues, Kjellmer (1964) developed an indirect method for its measurement in skeletal muscles. His method was based on inflating the blood vessels and measuring indirectly the venous pressure. He found that the tissue pressure is above zero and rises during exercise in relation to the amount of filtrate accumulated in the muscles. Against the concept of a negative pressure also, is Wiederhielm (1968) suggesting that the negative pressure in implanted capsules may result from the development of a semipermeable membrane. McDonald (1968) said there is no doubt that interstitial fluid exists outside the vascular tree and it has a volume which can be measured. Therefore, it must have pressure. Moreover, he added, the intercellular space in a horizontal body is at equilibrium with the atmosphere, and inside the gut the pressure must always be around atmospheric pressure, otherwise the "negative pressure" might result in the pulling of air into the interstitial space. This, he feels, makes it difficult to accept reports of a negative interstitial pressure.

In the experiments reported here what was estimated in actual fact by the needle-method is the sum of pressures exerted by all

tissue components, which is not only an interstitial fluid pressure, but also a solid tissue pressure. These compressible forces of the solid and semisolid elements of the tissue together with the fluid pressure form the total tissue pressure. It is this pressure which was estimated in the normal testis and in that having the tumour (Brown-Pearce carcinoma) and it is the same pressure which causes compression or occlusion of the tumour vessels resulting in focal necrosis. It may therefore be named appropriately the total tissue pressure. The high TTP of the Brown-Pearce carcinoma estimated in these experiments recalls the values estimated by Young and his co-workers (1950), Shivas (1955), and the findings of Gullino et al.(1964).

N.B. For convenience and simplicity in nomination, I shall refer to the "total tissue pressure" as the tissue pressure or the extra-vascular pressure.

Reference must be made to the slight fall in the values of the tissue pressure obtained in these experiments after Ismelin administration to the animals. This could be explained if the total tissue pressure rather than the interstitial fluid pressure was being measured, for otherwise we might obtain a marked fall in the values of the interstitial pressure after induction of hypotension. This is according to Starling's law of capillaries, in which a fall of the capillary pressure and of the interstitial fluid pressure can occur after lowering the arterial pressure in the host circulation (Starling, 1896 and Evans, 1968). My finding that the TTP of the Brown-Pearce carcinoma is much higher than that of the normal tissue after induction of hypotension in

the rabbit was an important point in planning the hypotensive experiments and in exploring the mechanisms of tumour necrosis.

4. Necrosis in tumours - mechanism of origin

Necrosis is a characteristic of many malignant tumours; its cause is arguable and probably several factors are contributory.

In considering the mechanism of necrosis in epithelial tumours, however, it seems feasible on the basis of the observations reported here, to explain necrosis as due to two mechanisms, namely, alteration in the local circulation as a result of hypotension, and compression of tumour vessels under relatively high extravascular pressure.

The question that arises is the possible rôle of other factors in the origin of tumour necrosis.

Young, Lumsden and Stalker (1950) and Shivas (1959) demonstrating the existence of special haemodynamics - chiefly a capillary venous hypertension in Brown-Pearce carcinoma, suggested that focal necrosis in tumours could be a result of collapse of tumour vessels under high tissue pressure, and Lewis (1927) who studied the vascular morphology of different types of rat tumours by India ink injection pointed out that necrosis was caused by obliteration of tumour vessels under the higher pressure of the growing neoplastic cells. He claimed that necrosis could occur as a result of continued multiplication of tumour cells exerting undue pressure on the capillaries or preventing in some way the further penetration of new capillaries into the areas produced by much multiplication. Absence of capillaries or failure to identify them in necrotic regions cannot be regarded as important in

this respect, because capillaries are often seen in necrotic tissue as demonstrated in this study (Figs. 11, 13 and 22) and supported by the findings of other workers as Goldacre and Sylvén (1962), Rubin and Casarett (1966) and Tannock and Steel (1969).

Some workers have failed to demonstrate capillaries in the necrotic parts of tumours and this can be explained as a result of inability of particulate matter to penetrate the vessels due to obstruction and stasis. This view is supported by the work of Goldacre and Sylvén who studied a large number of mouse and rat tumours by intravascular lissamine green injection. They were able to mark out regions not reached by blood-borne substances, and found that after the tumour reached a size of about 10 mm. in diameter, a central necrotic region appeared which did not colour green up to one hour after the intravenous dye injection, while the non-necrotic tissue was stained within two seconds. They assumed that the necrotic regions had no blood flow although they often contained patent vessels with intact red cells. Since the dye did not reach these vessels they decided that they were occluded somewhere. Histological studies showed a rich vascular network only at the periphery of the tumours, and in the necrotic regions all kinds of abnormal vessels were noted as sinusoidal channels, vessels lacking a membrane and others bordered by tumour cells.

The work of Rubin and Casarett is also of interest in relation to the microcirculation in tumours and the kinetics of necrosis. By

microangiographic methods, they studied two transplantable rat tumours, namely, Walker carcinosarcoma and Murphy lymphosarcoma. They assumed that necrosis is not a result of absence of capillaries between the tumour cords, or of decreased concentration of oxygen near the centre, but could be due to an alteration in tumour circulation rather than to the peripheral proliferations of tumour cords which are devoid of capillaries. It is remarkable that this important suggestion was not supported or followed by any experimental evidence.

The possibility of low oxygen tension as a main cause of cell death in tumours has been discussed by many workers and there is considerable disagreement on the subject.

Thomlinson and Gray (1955) in their histological studies of a large number of human lung cancers, assumed that the epithelial cords are supplied by capillaries from the periphery and that central necrosis occurs because of lack of capillaries in the centre, thus inducing a lower concentration of oxygen centrally as compared with the periphery. In these experiments, vessels were identified in the necrotic regions as in Thomlinson's sections and in those of Rubin and Casarett, indicating that those necrotic regions had been vascularised previously. Thomlinson and Gray also found that all cords of cells greater than 200μ in diameter have necrotic centres and came to the conclusion that whatever determined the onset of necrosis, the concentration of oxygen is lower toward the centre than at the periphery in the absence of capillaries among the tumour cells. Goldacre and Sylvén whose findings resembled those of Thomlinson and Gray, reported that cell death occurred at a distance close to the

oxygen diffusion length from the nearest blood vessel, but they gave evidence that tumour cells do not die in the complete absence of oxygen and anoxia is not sufficient to cause death in tumours.

Different mouse and rat tumours were studied by Tannock and Steel (1969, 1970). The tumour-bearing animals were exposed to sustained changes in the blood oxygen tension by maintaining the animals in a tank containing 10% oxygen in nitrogen for up to two weeks; the results were compared with control animals breathing air (21% oxygen). They came to the conclusions that low oxygen tension might affect cellular proliferation but have little effect on cell death in the rat mammary tumour; that the Ehrlich ascites tumour was even more resistant to hypoxia than solid tumours; and that in the mouse mammary tumour hypoxia was found to be a factor in the causation of cell death. They made further studies on the kinetics of tumour necrosis and found that despite the high vascular density, widespread foci of necrosis were seen in large tumours. They came to the conclusion that stasis in tumour blood vessels was the main cause of cell death in the rat mammary tumour. Further, by special technique, which employed ^{51}Cr-labelling of red cells and autoradiography, they were able to identify those vessels of the tumour which contained flowing or static blood. They suggested that stasis could be a result of occlusion of vessels by mechanical pressure, or thrombosis or the formation of capillary shunts nearer the periphery.

It seems therefore that although many authors accepted anoxia

as an important factor of cell death in tumours, others denied its importance in this respect (Warburg, 1956; Rubin and Casarett, 1966; IIjima et al.,1969 and Gullino et al., 1967). These authors have a general agreement that most tumour cells can survive in the absence of oxygen by switching over to anaerobic glycolysis, which is a metabolic characteristic of many tumour cells living in a hypoxic environment. These views were not explored by the present experimental work, although in the light of the observation reported here, oxygen deficiency attributable to absence of vessels can be excluded as a primary cause of necrosis in Brown-Pearce carcinoma because (1) vessels are often seen in the necrotic tumour with high concentration of red cells, thus indicating that necrotic regions were previously vascularised and had the same chance of oxygenation as the viable tumour, (2) islets of viable tumour cells remain in necrotic tissue. Therefore, it could be possible that anoxia or a reduction in oxygen tension in a tumour potentiates the onset of necrosis and, therefore it could be considered to be a contributory or ancillary factor to the mechanism that leads to necrosis in later stages.

Implicit in this research is the contention that tumour necrosis might be better understood and explained on the basis of alteration of physical factors of which hypotension is particularly important. The results of the experiments outlined above demonstrate the influence of induced hypotension in this respect in Brown-Pearce carcinoma growing in the rabbit testis. It is of course undesirable that the experiments were to some extent conditioned. It may be said

that the experiments indicate a possible way in which hypotension acts in this tumour. The evidence suggests that as a consequence of hypotension (1) the sinusoidal vessels dilate and the circulation through them is greatly slowed and may in fact cease, and (2) the relatively higher pressure in the tissues surrounding the sinusoids leads to their collapse, which greatly augments the hypotensive effect. Thus, in these two ways, induced hypotension may be said to accelerate and augment the development of necrosis in this experimental tumour.

Although it is clear from the work of Shivas and Gillespie (1969); Young et al. (1950) and others, that Brown-Pearce carcinoma might be expected to behave in this way wherever it is implanted, it is probable that the intratesticular site also influenced the results. The strength of the tunica albuginea although not forming a rigid case like the skull, is bound to have exerted a restrictive and compressive effect. Moreover, the testis is supplied solely by the spermatic artery which in this context is an end artery. There is no possibility of a collateral or peripheral circulation which in tumours in other situations may help to protect at least the peripheral parts of tumours from the necrotising effects of hypotension, but the fact that control tumours showed little necrosis can exclude that.

There is considerable evidence from the findings of the previous workers to show that purely physical forces in the tissue may play a dominant and primary part in the mechanism of necrosis in Brown-Pearce

carcinoma and possibly in other, malignant tumours. For example Younger and Algire (1949) were of the opinion that mechanical interference with the tumour blood supply was a factor in the slowing of circulation and subsequent occlusion of the capillaries which resulted in haemorrhage and necrosis in their experimental sarcomas, and Williams (1951) observed through the transparent chamber, that obliteration of the vascular supply by external tissue pressure was the main cause of necrosis in the V2 carcinoma grown in rabbit ears. Algire and Legallais (1951) found that there was a direct correlation between the fall in blood pressure and the reduction in the tumour vessels, and that tumour necrosis occurred when the hypotensive state was prolonged for several hours, but they explained the necrosis as a secondary effect resulting from tissue anoxis. Shivas and Gillespie (1969) were also of the opinion that systemic hypotension in the host circulation could cause a reduction in the vascularity of a tumour with subsequent necrosis.

A number of workers like Rubin and Casarett (1966) argued that pressure changes do not explain why one neoplasm develops necrosis upon attaining any significant size whereas others growing under similar conditions or in similar location show minimal necrosis. Although this might be the case in certain tumours, it still has its drawback for many reasons:

1. Each tumour has its own vascular pattern, as was stated by many workers, which means, each tumour has its own individual haemodynamics. Some tumours have afferent and efferent

vessels with a good deal of anastomosis, so that occlusion of some branches does not deprive the tumour blood supply completely, because the pressure in the main vessels is high enough to maintain adequate circulation throughout the tumour.

2. Once a tumour grows to a larger size, it forms rich anastomoses with the surrounding tissue; this means compression of its vessels does not severely affect the tumour viability, because the areas affected can have a blood supply from the surrounding tissue.

3. The rapidity of growth is quite an important factor. A more rapidly growing tumour may cause a sudden occlusion of its vascular supply with subsequent death of the tumour cells before newly formed vessels are originated. Once these vessels are formed, they are of minor use for the nutrition of the tumour cells because most of them have already died. While in a slowly growing tumour, the pressure exerted on the vessels causes a gradual deprivation of blood supply with or without occlusion of the vessels and at the same time newly-formed vessels originate from the existing ones, and take part in maintaining the tumour circulation with minimal necrosis.

The Brown-Pearce carcinoma grows slowly during the first week after implantation and necrosis is minimal, while at later stages it grows so rapidly that necrosis becomes inevitable.

5. Summary and Criticisms of the present work.

My results have shown:-

1. The Brown-Pearce carcinoma grows slowly in the rabbit testis for the first few days and then rapidly. It becomes palpable on the ninth or tenth day after implantation. During the third week it is large and reaches a size of 2-3 cm. in diameter. The Brown-Pearce carcinoma grows in the rabbit testis without necrosis up to the beginning of the third week. Thereafter and usually after the twenty-first day necrosis is commonly found. Its known behaviour with regard to necrosis has influenced the timing of the experiments. The experiments were completed before the twenty-first day so that spontaneous massive necrosis would not complicate the results.

2. The results of the tissue pressure estimations have shown that the tissue pressure of the Brown-Pearce carcinoma is much higher than that of the normal testis when the estimations were carried out on the twelfth and the nineteenth day after implantation. It was found also that the tissue pressure of the tumour increases with the lapse of time (Table 3, p. 27). In other words, there was a correlation between the size of the tumours and the tissue pressure readings. The estimations were made in normotensive animals and thirty minutes after induction of hypotension by intravenous injection of Ismelin. The results showed slight reduction in the values of the tumour tissue pressure readings after induction of hypotension. Despite this reduction in pressure the values remained substantially higher than those recorded in normal rabbits.

3. Episodes of hypotension increase the regularity and extent of necrosis. The experimental animals were rendered hypotensive 16 days after testicular implantation of the tumour when spontaneous necrosis is minimal or absent. The systolic blood pressure was maintained between 55-75 mm.Hg. over a period of 48 hours, and the side effects of Ismelin were minimal. The tumours were examined on the eighteenth day, while the controls were examined at the 16th day. There was a variation in the extent of necrosis in the experimental group: some tumours showed obvious widespread necrosis, others showed moderate and mild degrees of necrosis. There was also a considerable variation in the size of the tumours at 16-18 days after implantation as stated before. The experiment showed that the central portions of the tumour are most severaly damaged, while peripheral cells of the implanted tumour, adjacent to the blood supply of the normal testicular tissue survived.

From the experiments reported here it seems likely that tumour necrosis occurred after large doses (not lethal) of Ismelin which resulted in prolonged periods of hypotension.

4. Standard histological methods were used to investigate small blood vessels in the necrotic tumour. By the luxol-fast blue method, dilated vessels packed with red cells were demonstrated in the necrotic tumour portions but not in the viable tumour.

Stasis of blood in vessels is a prominent feature of necrosis. Evidence of vascular stasis was obtained indirectly by demonstrating fibrin deposition, which is frequently noted in the dilated vessels

of the necrotic tumour but not in the non-necrotic portions. The picro-Mallory staining method was used for this purpose.

From this it was inferred that stasis had occurred in the necrotic parts though no deduction could be made as to the time when the circulation had stopped.

Criticisms of the findings:

I. Role of homograft response

Since the Brown-Pearce carcinoma represents a homograft, it is possible that homograft reactions have contribution to the necrosis in the present study.

From different experiments it has been inferred that the agents or the mechanisms which destroy a tumour homograft are many. So far as the present investigation is concerned it is important to consider that a direct contact between sensitized lymphocytes and the cells of the Brown-Pearce carcinoma might have contribution to the massive necrosis seen.

It is well known that homotransplantable tumours can elicit a host response which can be studied over a period of time (Stuart and El Hassan, 1964, 1965; Thunold, 1968 and Baker et al., 1962).

The Landschütz Ascites tumour in mice was studied by Stuart and El Hassan (1964). The tumour elicited a host response manifested by round cell infiltration and splenomegaly. They studied this response (1965) over a period of time, after growing the tumour as a solid mass in the subcutaneous tissue. The tumour showed extensive

ischaemic necrosis and a peripheral chronic inflammatory reaction. They found a moderate infiltration of the tumour by plasma cells, lymphocytes, macrophages and mast cells, and increased phagocytic function after intraperitoneal inoculation. Their results showed an initial stimulation of R.E.S. function followed by a return to normal which was due to the suppression of the host response as a result of adoptive immunity.

Thunold (1968) examined the intraperitoneal cellular reaction and changes in splenic weight following the growth of his homotransplantable tumour EAC. He found a marked aggregation of lymphocytes to the tumour cells after the transplantation. He concluded that EAC initiates cellular reactions in the host similar to those seen in ordinary homograft. The morphological study also indicated that a process involving surface contact between tumour cells and the sensitized lymphocytes is a main point and may be of importance in the general tumour inhibitions; the tumour cells become surrounded by lymphocytes and are destroyed.

The available information in the experimental study which might raise the possibility that a homograft reaction has contributed to the necrosis in the Brown-Pearce carcinoma is obtained from histological examination in which some degree of lymphocytic infiltration, mostly around the tumours is seen. In my opinion it is entirely possible that homograft reactions have contributed to the tumour necrosis in the present study. However, this does not materially influence the

principal finding of enhanced necrosis in hypotensive animals although clearly it is a point which must be taken into consideration.

II. Cytotoxic effects of the hypotensive agent Ismelin.

It is possible that Ismelin has a cytotoxic action besides its hypotensive property. The central necrosis of the tumours found in my experiments might be analogous to the centrilobular necrosis caused by some hepatotoxic agents. Certainly it would be appropriate to use other hypotensive drugs and see if the same results are produced. However, no evidence of cytotoxic effects of Ismelin was found in the literature, and the results of the experiments showed that the central parts of the tumour are most severaly damaged in most cases, while peripheral cells of the tumour did not show necrosis. A significant cytotoxic effect of Ismelin is thought to be unlikely.

III. Histological techniques in studying tumour haemodynamics and vascular morphology.

Although histological techniques can be useful for studying the vascular morphology of tumours, they fail to provide information about the velocity or quantity of blood flowing through a tumour. In the present investigation three standard histological staining methods were applied, namely the luxol-fast blue, the picro-Mallory and the haematoxylin/eosin. The first is a special staining technique and was particularly useful in demonstrating small blood vessels difficult to see by other techniques especially by the injection techniques, which in many cases failed to demonstrate such vessels in the necrotic parts of the tumour. The second proved a convenient way of studying

stasis in the tumour vessels. The third used as a routine additional method. In the picro-Mallory staining method, evidence of vascular stasis in the tumour vessels was found histologically. However, no deduction could be made as to the time when the circulation had stopped.

CONCLUSIONS

Part 1

The conclusions which can be drawn from this part of the thesis are:-

1. The intravenous administration of Ismelin in pharmacological doses to rabbits induces an adequate fall of the blood pressure over a relatively long period without producing marked pathological changes in the animals.

2. The total tissue pressure of the Brown-Pearce carcinoma grown in rabbit testis and estimated by the "needle-pressure" method is much higher than that of the normal testis even when the systemic pressure is reduced by experimental means.

3. Hypotension induced and maintained for periods of 48 hours in rabbits bearing Brown-Pearce carcinoma is associated with necrosis in the tumour far exceeding its expectation as a natural event.

4. Hypotension and consequently necrosis in the tumour are possibly manifestations of the effect of Ismelin on the circulation and not due to any direct cytotoxic action on the tumour cells.

5. In Brown-Pearce carcinoma the necrosis is not merely a result of a deficient vascular supply, or lack of capillaries between tumour cords, or anoxia of tumour cells, but seems to be due to alteration in relative luminal and extravascular pressures with consequent compression of tumour vessels under a high total tissue pressure. The significance of these findings in relation to other tumours is discussed.

6. Further work is planned to explore the feasibility of extending the principle to tumour therapy, particularly that of disseminated tumour. Episodic reduction in the number of tumour cells, probably without emergence of resistant lines, seems a possibility.

PART 2

A HISTOLOGICAL STUDY ON THE GROWTH CHARACTERISTICS OF BREAST LUMPS IN WOMEN TAKING ORAL CONTRACEPTIVE HORMONES

1. Introduction

This part of the thesis was undertaken to describe the main histological features of a number of breast lumps in women taking oral hormonal contraceptives and to compare them with a group of controls without history of hormone administration; further to study the cases of carcinoma in each group; and to see whether any conclusions can be drawn on the effects of these contraceptive hormones on the breast morphology in general.

2. Development in Oral Contraceptive Hormones

The development of oral contraceptives was begun in 1952 by the synthesis of two progestational compounds namely, norethynodrel, the basic steroid in Enovid and norethindrone, the steroid in Orthonovum (Drill, 1965). Other orally active progestational compounds, some of which are more potent than the originals, have since been developed and evaluated.

The action of these contraceptive compounds is mainly to suppress ovulation by inhibiting the formation or the release of pituitary gonadotrophins and they may have oestrogenic or androgenic effects.

Numerous adverse effects of these compounds have been reported in about one-third of all women who start taking the pill, and the incidence varies somewhat with the dose of these drugs and the dose of oestrogen in particular.

These side effects can be summarised as:-

1. Gastro-intestinal symptoms as nausea, vomiting and headache etc. are believed to be due to the oestrogen.
2. Liver function impairments.
3. Thromboembolic and vascular changes.
4. Metabolic changes.
5. Occular, skin and miscellaneous changes which include dizziness, depression, fatigue, irritability, increased premenstrual tension and other effects which oestrogen-

progesterone can induce.

The adverse effects of these compounds on the female reproductive system and the breast morphology in particular are of special interest to the pathologist.

The contraceptive pills fall into two broad categories; the combination form contains both oestrogen and progesterone in each tablet. This gives virtually complete protection against pregnancy and they are in common use, while the other contains oestrogen or progesterone alone, combined with other steroids. The oestrogen used in the contraceptive pills is either ethinyloestradiol, or methyl ether (Mestranol). The progesterone in many of the pills is similar to ethyl testosterone, except that the methyl group attached to the carbon atom 10, has been replaced by a hydrogen atom (Norethindrone). The other progestational agents used are structurally similar.

Today millions of women use these two steroids only slightly modified in the contraceptive pills.

There is abundant evidence that administered oestrogen produces the same effect in the reproductive organs of a woman as occurs normally in the follicular phase of the cycle and the general biological affects seem to be identical. All oestrogens cause the cervix to secrete mucus, produce glycogen deposition and cornification of vaginal epithelium and stimulate the endometrium so that oestrogen withdrawal causes bleeding to occur.

The effects on the breast are not so well demonstrated, but there is no question regarding the general growth effects on the breast. Progesterone and oestrogen can produce clinical and morphological changes in the breast and as the hormonal oral contraceptives have similar hormonal properties, they may produce changes in certain women.

3. Review of Literature

Various histological changes in the female reproductive organs including cervix, endometrium and myometrium have been observed and described in women taking oral contraceptives (Candy and Abell, 1968; Charles, 1964; Kyriakos et al. 1968; Ober, 1966 and Taylor et al. 1967). On the other hand, comparatively little study has been made on the breast histology in those circumstances (Fechner, 1970 a, b and c, and Goldenberg, Wiegenstein and Mottet 1968).

Our knowledge concerning the influence of these contraceptives mainly comes from animal experiments in which the effects of these compounds on the breast were studied.

4. Study of the Histological Changes in Animal Breast Induced by Oral Contraceptive Hormones

In experimental animal models hormonal factors are of main importance for the development of mammary tumours (Bern and Nandi, 1961).

Kahn (1964) studied the effect of two types of contraceptives namely, Norethynodrel alone or in combination with Mestranol on the mammary gland of adult female rats. Both preparations caused extensive lobulo-alveolar development and increased secretion in the mammary glands after administration of a dose of 1.5 mg. for twenty-eight days. The work of McCarthy (1965) is of interest. She studied the effect of steroid antifertility agents on the induction of mammary gland tumours in animals, and found an increased incident of cancer in Sprague-Dawley rats treated with 0.25 mg. of Enovid or Norlestrin daily for ten days prior to a single feeding of 8 mg. DMBA, while no difference was found when a larger dose of 1 mg. daily of the antifertility compounds was used. Thus she concluded that the induced hormonal change did in some way increase the likelihood of malignant changes in the mammary epithelium. On the other hand, a decreased incidence of induced mammary cancer in rats was reported after feeding 0.3-3.0 mg. of Enovid daily for forty-five days beginning ten days before single administration of 15 mg. of DMBA (Weisburger et al., 1968). Stern and Mickey (1969) studied the effect of contraceptive steroids on the mammary gland tumour in female rats

following a single dose of the carcinogenic DMBA in one group. Tumour growth obtained in one group of rats not receiving DMBA is of interest, because of the possibility that a hyperoestrogenic state resulting from prolonged use of steroid antifertility compounds might increase mammary cancer, as was stated by Hertz and Bailar (1966).

5. Study of Morphological Changes in Human Breast Induced by Oral Contraceptive Hormones

The effects of hormonal oral contraceptives on the morphology of human breast in spite of being a matter of biological importance, has received little study. Florid benign or preneoplastic and neoplastic epithelial proliferations (epitheliosis) are of significance to the pathologist, because they give precise knowledge of the effect of hormones on the breast histology and they represent at least potential transitional stages on the path to breast carcinoma.

Gregg (1966) reported on a case of galactorrhoea with a fibroadenoma in the breast of a 23 year old girl who was taking norethynodrel with Mestranol (Enovid) in a dose of 5 mg. initially, then she increased the dose to 20 mg. Similar cases of galactorrhoea were reported by other workers (Schachner, 1966 and Yaffee, 1966).

A morphological study of four cases of fibroadenoma in women taking the contraceptive pills has been made by Goldenberg, Weigenstein and Mottet (1968). They described bizarre epithelial proliferation and increased secretion in these cases. Although there was no evidence of carcinoma in any of them, they stated that hormonal therapy may have led to the abnormal proliferative changes.

Similar changes have been described recently by Fechner (1970 a and b), who stated that the proliferative changes in fibroadenoma and fibrocystic disease of the cases he studied carried no specific

significance in regard to the patients taking the pill, as similar changes were observed in their controls. On the other hand, Shipman (1964) reported on two cases of breast carcinoma in two young women taking oral contraceptive hormones. He speculated further on the effect of hormones on the development of breast cancer, suggesting that hormonal compounds should be discontinued in such cases. He stated that whether or not these hormones favour the origin, or the growth, or the viability and persistence of secondaries is not sure, but as far as they cause an increased blood flow to the breast in the premenstrual phase, it might be advisable to discontinue the drugs in suspected cases.

It seems obvious from the general review of the literature that hormonal factors (oral contraceptives) do play a part in the abnormal morphology of certain breast conditions. The study of such histological alterations in breasts in some detail is an important object at the present time.

6. The Object of the Study

The aim of this study is to compare the histological appearances in the breasts of forty-five women taking oral contraceptives with a similar number of controls and to draw conclusions.

7. Materials and Methods

The case summaries and the sections from ninety patients with breast lumps and from whom tissue biopsies were received in this Department between 1965 and 1970 were reviewed. On average, six sections from each case were examined. Of these, forty-five were women taking oral contraceptives. The other forty-five were women not taking hormonal therapy and were used as controls. All cases of fibroadenoma, fibrocystic disease and carcinoma were tabulated (Table 7), and the detailed findings of each case are given in the appendix. The histological sections from the control group and the hormone group were examined without knowledge of the hormone therapy.

Fibroadenoma was diagnosed when the mass of tissue was mainly a fibroadenoma. Small foci of fibroadenoma found incidentally with other lesions were not included. At the same time fibrocystic disease was diagnosed when there were two or more of the following abnormalities:-

1. Cysts (microcysts or macrocysts).
   microcysts: dilated ducts which can be recognised microscopically.
   macrocysts: grossly dilated ducts which can be recognised macroscopically.
2. Epitheliosis: multiplication of epithelial cells within duct or ductule, including solid, papillary or multi-layer cell patterns.
3. Apocrine metaplasia: duct or cyst of normal size which is lined by typical granular, pinkish columnar epithelium with papillary projections among them.

4. Fibrosing (sclerosing) adenosis: proliferation of terminal ducts which become compressed and distorted by the proliferating fibrous tissue within the lobule.

Two other terms need clarification:-

Secretion:- This term was applied to indicate the presence of secretory material within the lumina of ducts and acini of the cases examined as well as the finding of secretory vacules in the cytoplasm of the epithelial cells, by which the secretory activity of each group can be evaluated and compared with the other group.

Adenosis:- An increase in the number and size of lobules which is often histologically indistinguishable from that found in the early glandular increase of pregnancy.

Epithelial hyperplasia (epitheliosis), seen within an existing duct of fibroadenoma, was included under epitheliosis (Tables 8 and 9).

The histolgocial features, incidence of each abnormality and gradation of each category, are summarised in Tables 8 and 9, and Text-Figure 5.

Sections from each group were reviewed to pick up any trends not considered in the first study.

TABLE 7

Incidence of Fibroadenomas, Fibrocystic Diseases and Carcinomas Compared in a Total of Ninety Cases

Group	No. of Cases	Fibroadenoma	Fibrocystic Disease	Carcinoma
Contraceptive	45	14 (31.1%)	27 (60%)	4 (8.9%)
Control	45	17 (33.6%)	27 (60%)	1 (2.2%)
Total	90	31	54	5

TABLE 8

Incidence, Gradation and Main Histological Features of Forty-Five Cases of Breast Tumours in Patients Taking Oral Hormonal Contraceptives.

Compare with Table 9

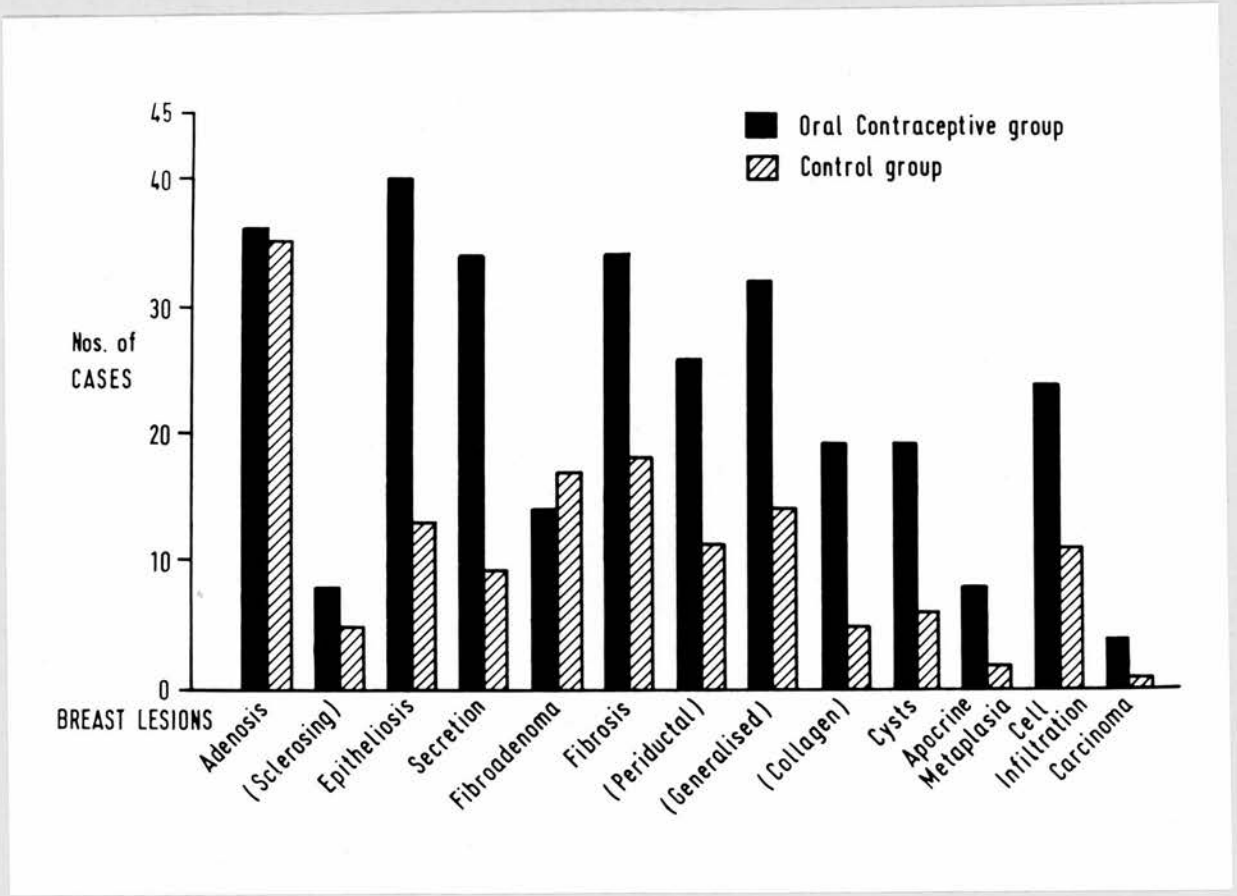
Histological Abnormality	No. of Cases	%	Gradation 1		
			Slight	Moderate	Severe
Adenosis	36	80	13	22	1
(sclerosing)	8	17.8	8	0	0
Epitheliosis	40	88.9	16	15	9
Secretion	34	75.5	20	10	4
Fibroadenoma	14	31.1	1	6	7
Fibrosis	34	75.5	14	17	3
periductal	26	57.8	18	6	2
generalised	32	71.1	24	8	0
collagen	19	42.0	16	3	0
Cysts	19	42.0	11	6	2
Apocrine metaplasia	8	17.6	7	1	0
Cell infiltration	24	53	19	4	1
Carcinoma	4	8.9	1	3	0

1. Each category was graded as absent, slight (single duct involved), moderate (2-4 ducts involved) or severe (5 or more ducts involved).

TABLE 9

Incidence, Gradation and Main Histological Features of Forty-Five Cases of Breast Tumours in Patients Having No History of Hormonal Therapy

Histological Abnormality	No. of Cases	%	Gradation		
			Slight	Moderate	Severe
Adenosis	35	77.7	13	21	1
(sclerosing)	5	11.1	5	0	0
Epitheliosis	13	28.9	7	6	0
Secretion	9	20	8	1	0
Fibroadenoma	17	33.6	4	11	2
Fibrosis	18	40	14	4	0
periductal	11	24.4	11	0	0
generalised	14	31.1	14	0	0
collagen	5	11.1	5	0	0
Cysts	6	13.3	6	0	0
Apocrine metaplasia	2	4.4	2	0	0
Cell infiltration	11	24.4	10	1	0
Carcinoma	1	2.2	0	0	0



Text Fig. 5 A histogram showing the incidence of different breast lesions in patients taking oral contraceptive hormones compared with a group of controls.

8. Results and Observations

The age group of patients taking hormonal therapy ranged from 19-44 while the controls ranged in age from 17-47.

The duration of the hormonal intake ranged from six months to four years before surgery was carried out. In 8 patients, the preparation was stated in the case summary. In 2 patients, it was predominantly oestrogenic in its effect (Enovid,[1] Ovulen[2]), and in 6 patients it was progesterone-like in effect (Norinyl,[3] Norlestrin,[4] Anovlar,[5] Gynovlar,[6] and Lyndiol[7]).

In the remaining 37 cases it was stated only that the patient was taking oral contraceptives without mentioning the specific preparation. All cases studied were from premenopausal women.

The percentages of fibroadenomas, fibrocystic diseases and carcinomas in each group are listed in Table 7.

Morphological Observations

The epithelial characteristics in each group were analysed and compared with the other group. Although many biopsies in both groups had ducts lined by a single layer of normal resting epithelium (Fig. 28), the number of cases showing epitheliosis was greater in the oral contraceptive group, where it occurred in forty cases and

---

1. Norethynodrel and Mestranol.
2. Ethynodiol diacetate and Mestranol.
3. Norethisterone and Mestranol.
4. Norethisterone acetate and ethinyloestradiol.
5. Norethisterone acetate and ethinyloestradiol.
6. Norethisterone acetate and ethinyloestradiol.
7. Medraxyprogesterone acetate and ethinyloestradiol.

only in thirteen cases of the control breasts (Tables 8 and 9). Epithelial proliferations in ductules and ducts of fibroadenoma cases of each group were included in these figures (Figs. 29 and 30). The epithelial cells from the oral contraceptive group showed some pleomorphism varying from flat or cuboidal to columnar type arranged in many layers and frequently displaying a papillary pattern (Figs. 31, 32 and 33). Many ducts were dilated and filled completely with the proliferating cells showing a cribriform or a solid pattern (Figs. 34 and 39). In many places the florid appearance and the disorderly pattern were so obvious that they were suggestive of malignancy, but lacking the atypia of sufficient extent to consider a possibility of malignancy. Careful high-power examination revealed uniform cellular pattern, though carcinoma <u>in situ</u> was not excluded in some cases (Figs. 35 and 41). Control biopsies showed no abnormal features apart from slight epitheliosis in a few cases (Table 9), but the type of cellular proliferation and general appearance were benign (Figs. 36, 37 and 38).

9. Report of a Case of Marked Epitheliosis with Papillomatosis

A case of marked epitheliosis with papillomatosis was identified from the oral contraceptive group.

A 22-year old married woman had a lump in the right breast for about three-and-a-half years. The nodule increased in size over the last two months before admission to hospital in February, 1966. It was not painful and there was no alteration in size during the menstrual periods. No bleeding or discharge from the nipple was observed. She had a previous history of rheumatic heart disease in 1965. She had been taking oral contraceptive hormones (Gynovlar) for the last year before admission. The clinical diagnosis was fibroadenoma.

Morphological Examination

The sections examined showed an obvious dilatation of glands, ductules and ducts, some of which were lined by apocrine-type epithelium. There was marked epitheliosis of severe degree with papillomatosis, but there was no invasion of the basement membrane, although in some areas the dilated structures appeared to be fused with each other. The epithelial cells showed some pleomorphism varying from flat or cuboidal to columnar-type with few mitotic figures, but still there is insufficient atypia to consider the case as malignant. A dense periductal inflammatory infiltrate was seen with many ducts containing abundant secretion and small fat-containing cells (Figs. 39, 40, 41 and 42).

Secretion was a prominent feature in the oral contraceptive group, occurring in thirty-four cases, but only in nine of the control cases. This was shown by the presence of secretory material within the lumina of the ducts and acini as well as through the findings of secretory globules in the cytoplasm of the epithelial cells (Figs. 32 and 43). Secretory activity was not a prominent feature of the control cases.

Both groups showed an increase in the fibrous tissue stroma with various degrees of stromal pattern ranging from relatively acellular collagen to a loose cellular stroma (Figs. 28, 31 and 36).

Apocrine metaplasia was more common in the oral contraceptive group, occurring in eight cases, but occurring in only two of the control group. An interesting observation is that apocrine metaplasia was only occasionally seen in fibroadenoma cases, but was commonly seen in fibrocystic disease, mainly in association with epitheliosis, and sometimes exhibited a papillary pattern (Figs. 33 and 39).

Microcysts were found in greater extent in the oral contraceptive group, occurring in nineteen cases, but occurring in only six of the control cases.

Cellular infiltration was seen in twenty four cases from the oral contraceptive group and eleven cases of the controls. This consisted mainly of lymphocytes, plasma cells and few histiocytes (Figs. 32, 38 and 47).

In many cases of the oral contraceptive group there was a distortion of the lobular pattern with disorderly proliferation of ducts and acini of different shapes and sizes and many of these dilated ducts showed infolding by the proliferating stroma.

There is a possibility that some of the cases examined represent carcinoma in situ (Figs. 35, 41 and 46). It should be emphasised, therefore, that the presence of active mitosis, even in the absence of cellular atypia and invasion of basement membrane must rouse a certain degree of suspicion that the lesion in question may represent a stage in the development of malignancy.

10. Report of Five Cases of Carcinoma

Among the ninety cases of breast lumps reviewed, five cases of carcinoma were identified. Four cases were of women taking the pill and the fifth case was from the controls. The main histological features of these cases are summarised in Table 10.

The microscopic features were similar in all cases, showing epitheliosis with fibrocystic disease, intraduct and invasive carcinoma (Figs. 44, 45 and 46).

TABLE 10

Age Group and Main Histological Features

of Carcinoma Cases Reviewed in this Study

Serial Nos.	Age	Histology Summary
Case 3	36	Epitheliosis with fibrocystic disease + adenocarcinoma
Case 13	28	" " " " "
Case 26	38	Intraduct carcinoma with invasive carcinoma + fibrocystic disease
Case 33	28	Intraduct carcinoma with invasive carcinoma + fibrosis
Case 25 (control)	43	Intraduct carcinoma with invasive carcinoma + fibrocystic disease

11. Discussion

The incidence of fibroadenomas expressed as a percentage was 31.1% in the oral contraceptive group, compared with 33.6% of the control group. The nearly identical incidence and the fact that there has been no obvious increase in the incidence of fibroadenoma might signify that oral contraceptives are not a real cause of new fibroadenomas. An acceptable explanation, however, is that patients with fibroadenomas which appear during oral contraceptive therapy are patients who already had fibroadenomas and that the hormonal therapy stimulated the pre-existing lesion to become clinically distinguishable. The microscopic appearance of biopsies in each group is more important than the incidence of fibroadenomas.

Fibroadenomas from patients taking oral contraceptive hormones were qualitatively distinguishable from the control tumours. It was noticed that while the control fibroadenoma exhibited no abnormal features, bizarre epithelial proliferation (epitheliosis) and marked secretory activity were commonly observed in many cases of fibroadenomas from patients taking oral contraceptives. Somewhat similar epithelial proliferations were reported in four cases of fibroadenoma from patients taking oral contraceptive hormones (Goldenberg et al, 1968). Weigenstein and Mottet Adenomatous-like lesions in the form of acinar proliferation similar to that seen in fibroadenoma removed during pregnancy and lactation (Le Gal, 1961) infrequently observed in non-pregnant women were seen in this series (Fig. 47). Similar

acinar hyperplasia with secretion was reported in two cases out of a large number of patients taking oral hormonal therapy (Fechner 1970b).

A few cases of galactorrhoea have been reported in patients taking oral contraceptives (Gregg, 1966; Schachner, 1966 and Yaffee, 1966), although it is not clear whether this is due to the direct effect of the hormones on the breast, or on the hypothalamus. Acinar hyperplasia with abundant secretion reported in this series of women taking hormonal therapy might be a morphological counterpart of galactorrhoea in those patients taking the hormonal treatment.

The incidence of epitheliosis, expressed as a percentage was 88.9% in the oral contraceptive group. A virtually smaller incidence of 28.9% has been obtained in the breasts of control women.

The fact that there is a significant increase in the incidence of epitheliosis, gives a strong claim that oral contraceptive hormones could cause epitheliosis in an already existing benign breast tumour.

The incidence of fibrocystic disease was identical, making 60% in each group. It might be possible therefore that hormonal therapy causes a greater incidence of epitheliosis in an already existing fibroadenoma or fibrocystic disease.

The identification of a case of marked epitheliosis with papillomatosis is a striking observation in this study. A similar finding in a young woman who had been taking hormonal contraceptives was illustrated by Ackerman and Taylor (1969) who raised the question of its relation to the hormonal treatment.

The main purpose of this study was to find whether the frequency of epitheliosis was greater in patients taking hormonal contraceptives than in those having no history of hormonal therapy. This concern was largely affirmed on the possible association between epitheliosis as a completely pathological condition and cancer.

All the proliferative lesions discussed here, can be seen from time to time to form the starting point of cancer, and when the cancer has obliterated its site of origin by its own invasive growth, multifocal changes in the remainder of the breast tissue may suggest an origin from cystic or proliferative lesions.

The identification of four cases of carcinoma in the vicinity of cystic hyperplasia in young and middle-aged women taking the hormonal therapy in the present study may raise the query of their relation to the hormonal treatment, and further investigation of such cases is essential.

The relationship between cystic hyperplasia and carcinoma of the breast has been confirmed by innumerable authors, such as Cheatle and Cutler, 1931; Dawson, 1933; Willis, 1967; Bonser et al., 1961 and Gallager and Martin, 1969.

Bonser et al, (1961) stated that although an overwhelming majority of opinions favour a positive relationship between cystic hyperplasia as precancerous condition and infiltrating carcinoma, there is no knowledge of the percentages on which they occur together, although cystic disease and epitheliosis either separately or together are

found frequently in the cancerous breast. She further commented on the histological relationship between cystic hyperplasia and carcinoma in human breast, stressing that the best histological evidence in support of this relationship is the demonstration of the actual origin of the neoplastic cells in the cysts and the breakout of these cells into the surrounding breast stroma, which can be seen when breasts with early infiltrating cancer are studied. Gallager and Martin (1969) were also of the opinion that epitheliosis and cystic hyperplasia are stages in the developing carcinoma. They made a mammagraphic histological study of breasts of patients suspected to have breast carcinoma and found alteration of considerable extent present in the duct epithelium of each breast containing cancer, ranging from simple hyperplasia to hyperplasia with cellular atypia to intraduct carcinoma. This seems strong support of the view that epitheliosis and carcinoma are causally related and the two phases are a continuous process.

The morphological changes in the breasts of women taking oral hormonal contraceptives reported in this study, carry a large similarity to the morphological changes reported in the cervix (Candy and Abell, 1968; Taylor et al. 1967) and endometrium (Ober, 1966).

The illustration of similar patterns of prominent changes in the breast epithelium in this study, might provide a basis to support a definite relationship between oral contraceptive hormones and breast diseases, and further study is needed to clarify more of the effects of these hormonal compounds on the human breast.

12. Evaluation of Possible Relation Between the Incidence of Breast Cancer and the Pill.

To decide whether or not there is a relation between the induction of cancer and oral contraceptives requires a large statistical study. It has been calculated by Hertz (1969) that to undertake such study, samples of 15,000 - 20,000 young women would be required in order to demonstrate a statistically valid two-fold increase in breast cancer.

The published data available on the incidence of cancer for the age group presently under consideration is limited in a number of studies made. Such limited data fail to provide an adequate epidemiological basis for a satisfactory decision on the possible carcinogenic effects of the pill.

Allen (1969) considered that the constantly increasing number of cases of breast cancer is because of the increasing population and longevity, but the incidence rate per 100,000 population has remained relatively unchanged despite the extensive use of oestrogen for more than thirty years and the widespread use of the pill for more than ten years. Fechner (1970c) also found no evidence of association between hormone and breast carcinoma in a small group of 10 cases of breast cancer in women under 35 years of age of which 5 were on the contraceptive pill. His study presented 4.4% of the total 223 breast cancers seen during the same period of time in the Methodist Hospital in Houston. Other recent studies in the U.S.A.

(Horseley et al., 1969 and Goldenberg Mottet and Wolff, 1968) showed that the frequency of carcinoma of the breast in women less than 35 years of age remains at 5 per cent of all breast cancer in U.S.A. Hospital population, which is similar to the proportion of breast cancer in young women before the availability of the pill. No such studies were carried out in Britain.

Hertz (1969) has made an interesting point that all human carcinogens exhibit a prolonged latent period and that we may have only recently entered the period for initial carcinogenic effect of the pill, since the longest exposure of an individual to oral contraceptives dates back to about 15 years.

Finally it should be noted that with the wide use of oral contraceptives and the great frequency of certain breast and uterine neoplasms, it is common to find that a history of hormone intake will often coincide with such common lesion in the same patient especially in women under 35 years of age who make the major bulk of the oral contraceptive users. Therefore, the possibility of coincidence should be considered before assuming a rising incidence rate in such cases.

In conclusion there has been no clear indication of increase in breast cancer since the use of the pill became common.

13. Summary and Conclusions

Part 2

Morphological study of ninety cases of breast tumours with breast biopsies sent to this Department was carried out. Forty-five cases were of women taking oral hormonal contraceptives. Slides from these cases were compared with slides from another forty-five biopsies of women from the same age group but having no history of hormonal therapy.

The incidence of fibroadenoma, fibrocystic disease and carcinoma was studied and the main microscopic features were described and compared with the other group.

Epitheliosis was a prominent feature in the breasts of women taking hormonal therapy. A case of marked epitheliosis with papillomatosis was described. The lack of cellular atypia and invasion of the basement membrane excluded the diagnosis of malignancy. Four cases of carcinoma in young and middle-aged women taking the oral contraceptive hormones were described. The possible relationship between epitheliosis and carcinoma was discussed and the possibility of certain changes in the breast provoked by these hormones was raised. The degree of histological changes and the amount of atypia might depend on the dose, duration of treatment and the ratio of oestrogen to progesterone in these compounds, and further investigation is needed.

Fig. 28 Fibroadenoma from a 22 year old woman taking oral contraceptives, showing cystic dilatation and increase in the periductal and the generalised fibrous tissue. Note ducts lined by one layer of epithelium while others show epithelial proliferation.

H. and E. x 60

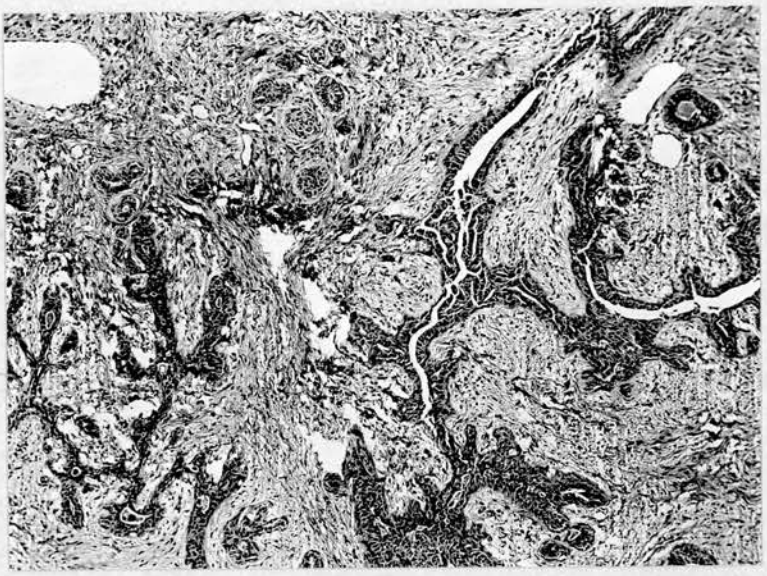


Fig. 29 Fibroadenoma from a 25 year old woman taking oral contraceptives, showing epithelial proliferation within the ducts. Note haphazard distribution of ducts and acini with relatively cellular stroma.

H. and E. x 60

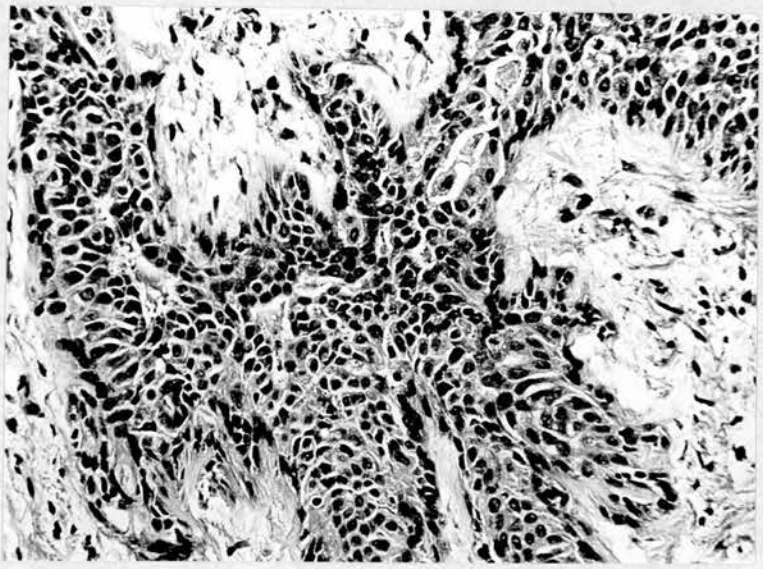


Fig. 30 Details of Fig. 29. Epitheliosis within dilated duct of fibroadenoma. Note the cells almost completely fill the lumen. They are small and regular and lack atypical features.

H. and E. x 275

Fig. 31 Hyperplastic epithelial proliferations within ducts and ductules of fibroadenoma from a 19 year old woman taking the pill. Note highly cellular stroma.

H. and E. x 60

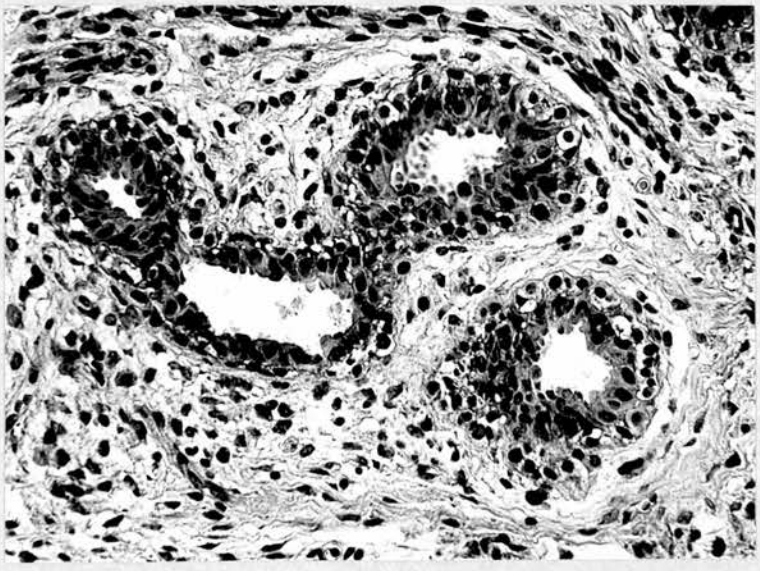


Fig. 32 Details of Fig. 31. Hyperplastic epithelium within ductules of fibroadenoma. Note the cells are cuboidal or columnar arranged in several layers and showing secretory activity. The myoepithelial cells can be seen in association with the ductules.

H. and E. x 275

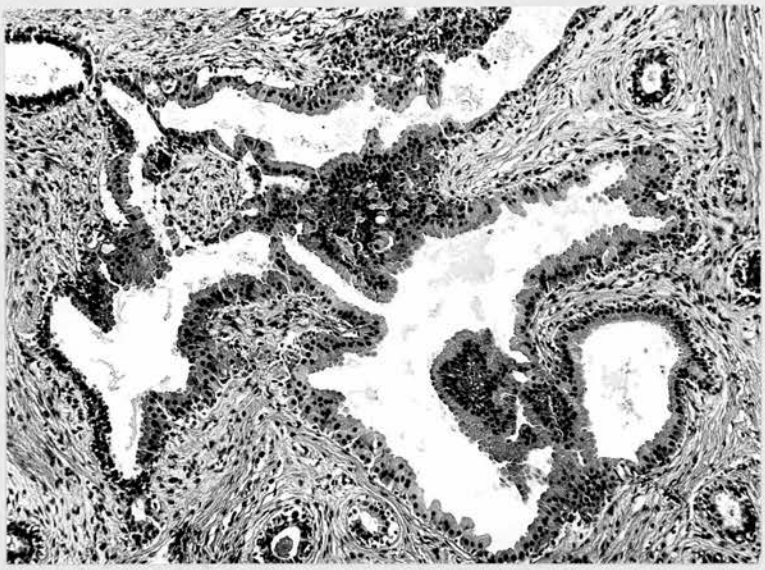


Fig. 33 Dilated ducts of fibroadenoma from a 24 year old woman taking the pill. Note epitheliosis with apocrine metaplasia within the large ducts and papillary projection into the lumina.

H. and E. x 100

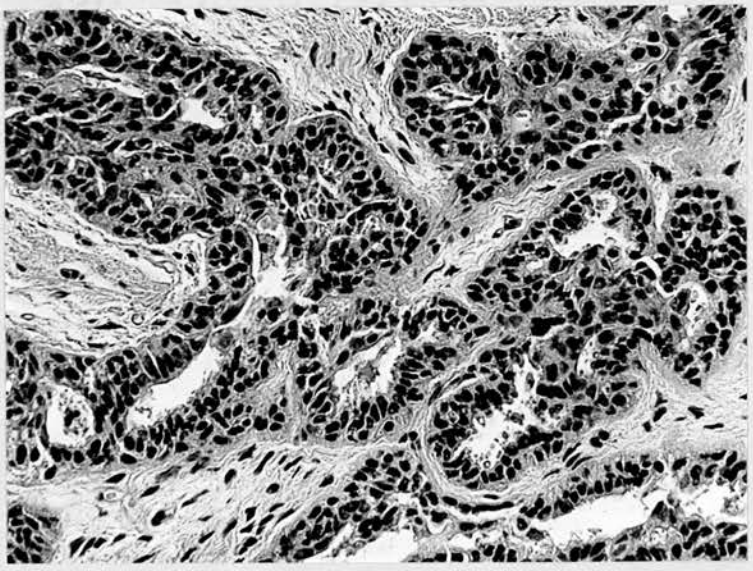


Fig. 34 Epitheliosis filling many ducts. Note the hyperplastic appearance of the epithelial cells and their lack of cellular atypia.

H. and E. x 275

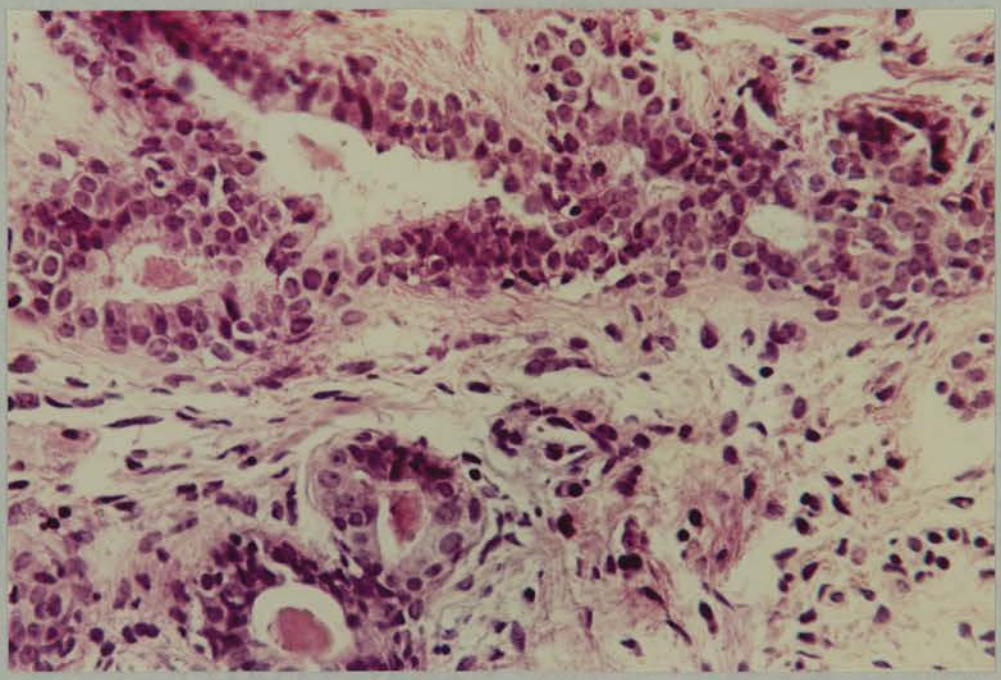


Fig. 35 Hyperplastic epithelium. Note many mitotic figures and increased secretory activity. The high cellularity and disorderly pattern may give an impression of malignancy. The myoepithelial cells can be seen in association with the ductules.

P.A.S. x 450

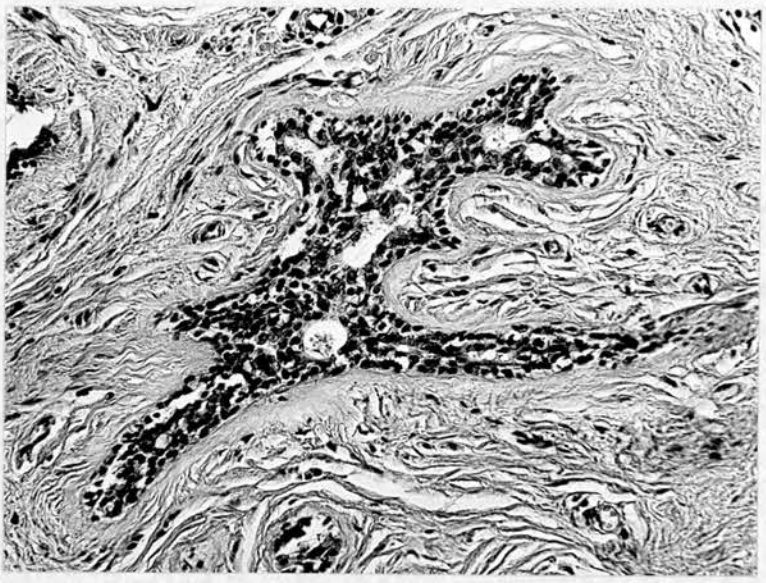


Fig. 36 Control fibroadenoma in a 27 year old woman. Note epithelial proliferations are benign and showing no abnormal features. (Lump 10 years' duration).

H. and E. x 200

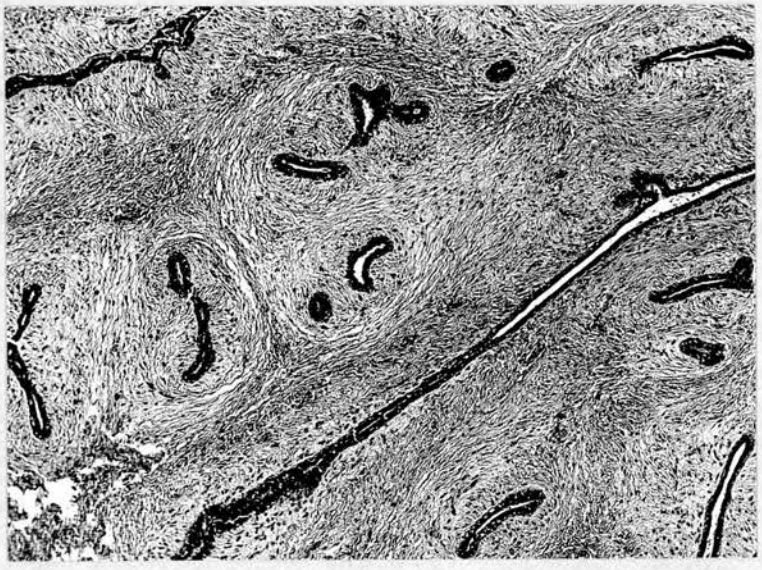


Fig. 37 Control fibroadenoma from a 19 year old woman. Note no abnormal epithelial changes and stroma has quite a benign appearance.

H. and E. x 60

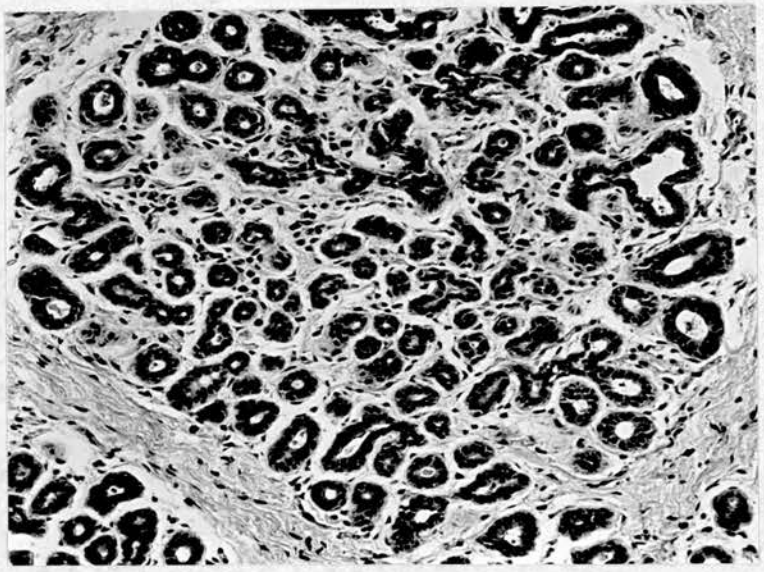


Fig. 38 Control fibroadenosis in a 32 year old woman. Note many acini lined by a single layer of resting epithelium, with secreting material in lumina. The stroma is fairly cellular. Note the histological similarity of the section to that of early normal pregnancy.

H. and E. x 150

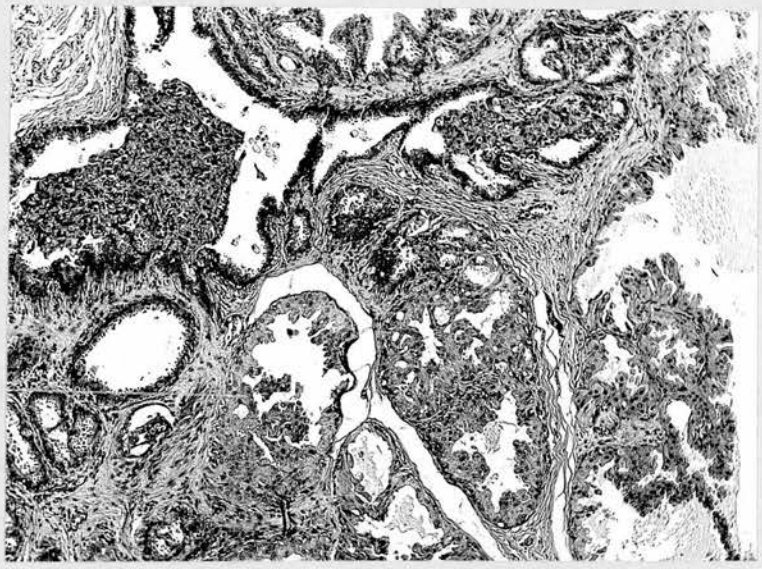


Fig. 39 Marked epitheliosis with papillomatosis in a 22 year old married woman taking oral contraceptives. Note many dilated ducts and ductules partially filled with proliferating cells. In many places the epithelium shows apocrine metaplasia and prominent papillary projections into the lumina.

H. and E. x 60

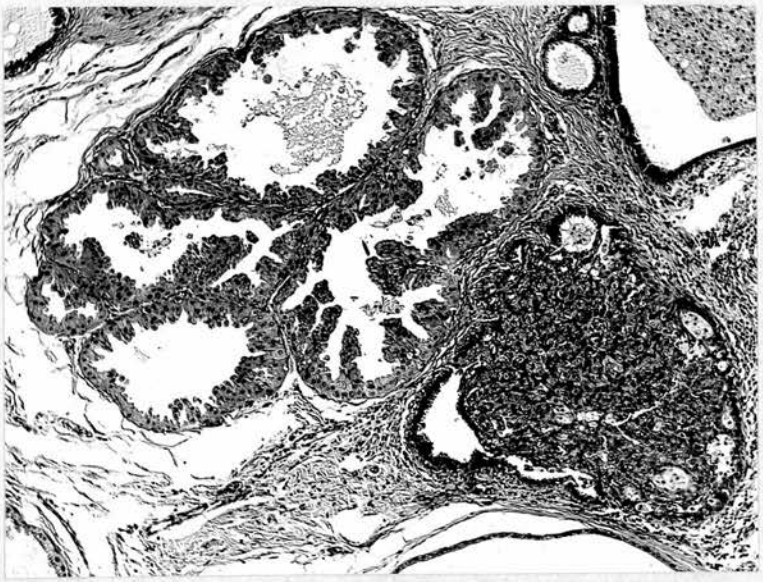


Fig. 40 Proliferating cells almost completely fill the lumen. Note the adjacent ducts are lined by apocrine epithelium showing papillary projections in many places. The high cellularity and the tendency of these structures to fuse together give an impression of malignancy.

H. and E. x 70

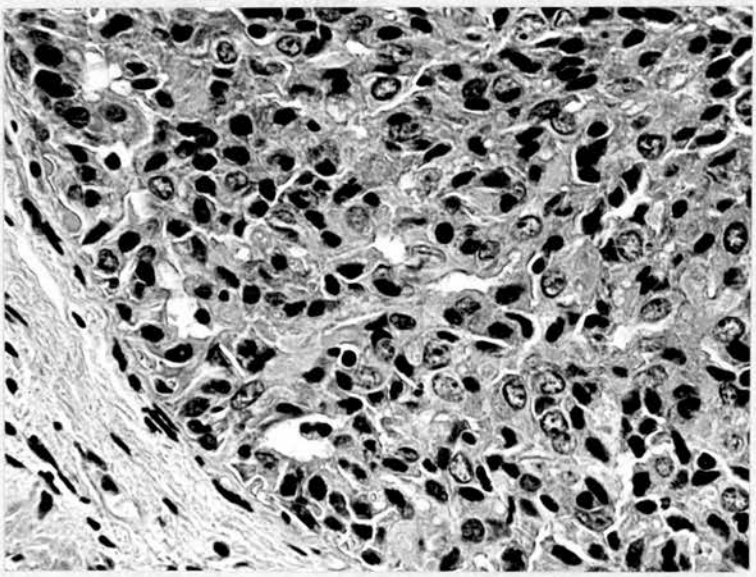


Fig. 41 Details of Fig. 40, showing epitheliosis within large duct. Note the florid appearance of the epithelium with many mitotic figures and lack of cellular atypia or invasion of the basement membrane, though carcinoma <u>in situ</u> is not excluded.

H. and E. x 500

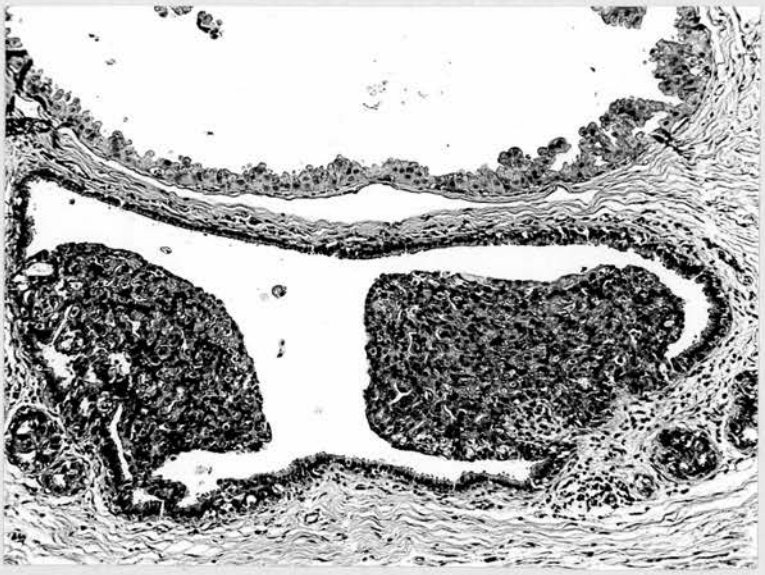


Fig. 42 Two papillomatous projections into a dilated duct. Note benign epithelial proliferations. The duct at the upper part is lined by apocrine epithelium.

H. and E. x 90

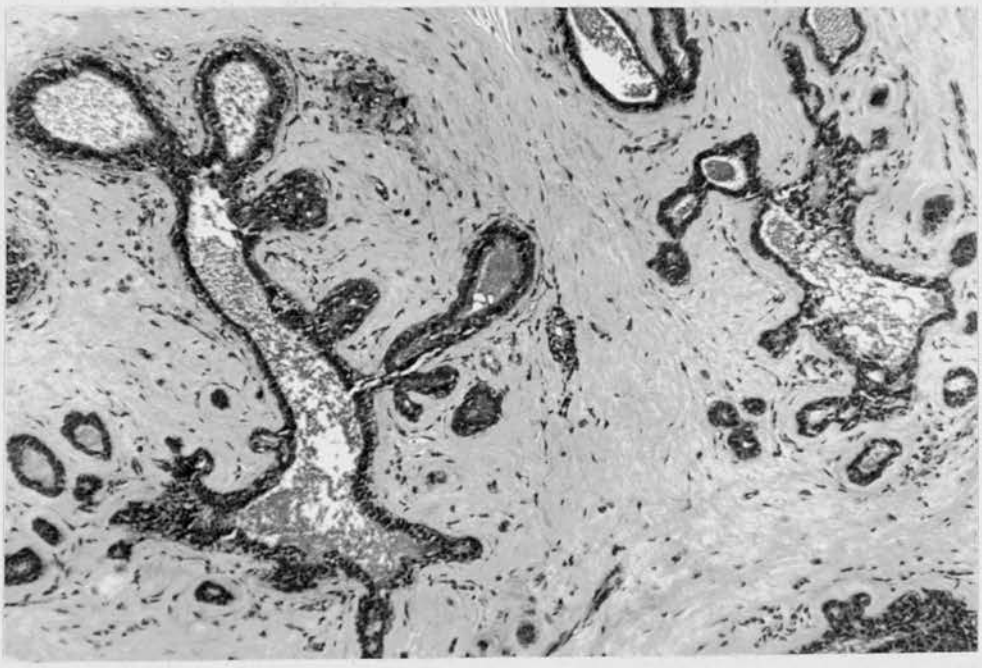


Fig. 43 Dilated ducts from a fibroadenoma in a 22 year old woman (Fig. 28) who was taking oral contraceptives. Note conspicuous secretory activity.

Alcian blue x 120

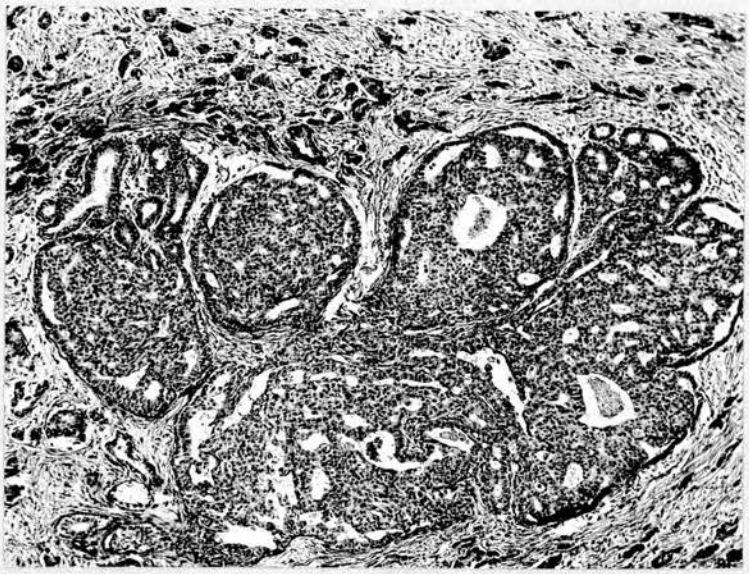


Fig. 44 Cluster of ducts showing non-invasive intraduct carcinoma from a 38 year old woman taking oral contraceptives. Note the tumour has a cribriform pattern. An invasive carcinoma is present elsewhere in the section.

H. and E. x 60

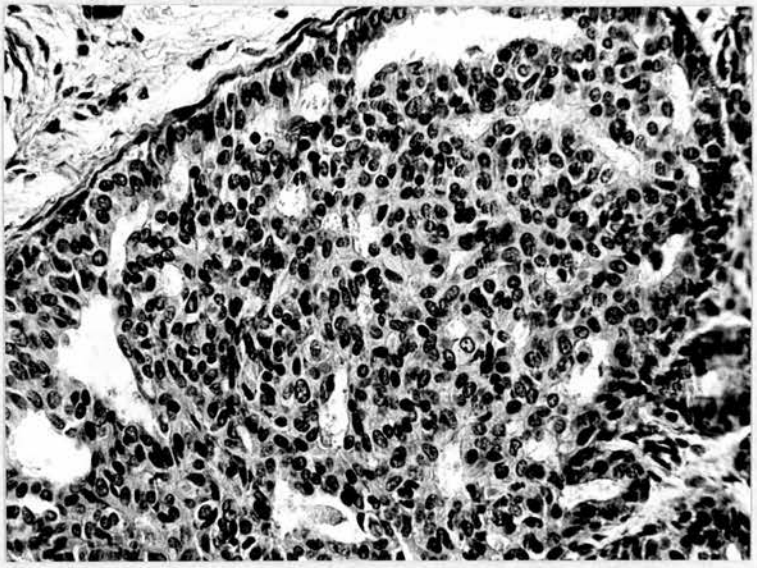


Fig. 45 Details of Fig. 44 showing part of a duct containing tumour cells. Note many mitotic figures and the tumour is not invading the basement membrane.

H. and E. x 275

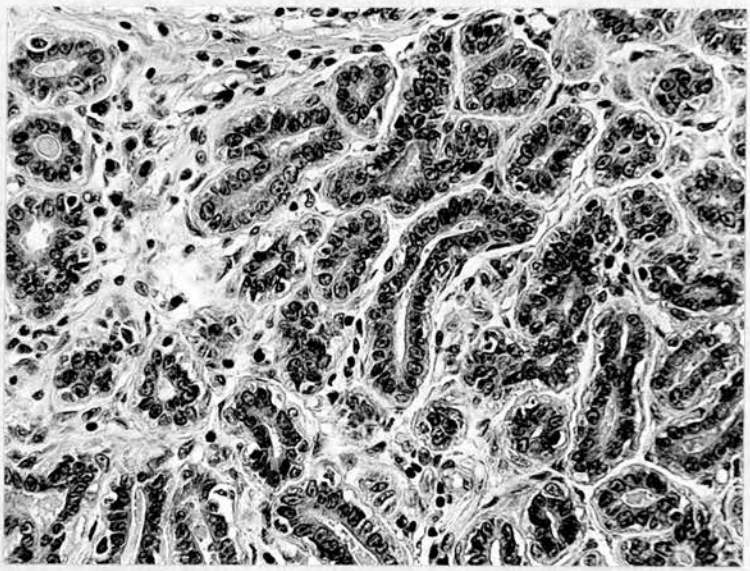


Fig. 46 Hyperplastic adenosis in the vicinity of the carcinoma illustrated above. Note hyperplasia with cellular atypia and many cells showing mitotic activity. The possibility of carcinoma <u>in situ</u> should be considered in this case.

H. and E. x 275

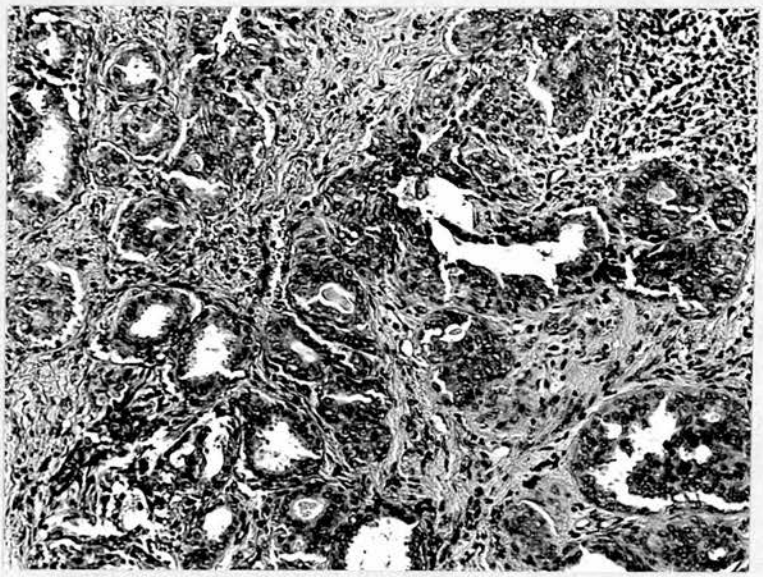


Fig. 47 Acinous proliferations in the breast of a 25 year old woman taking the pill. Note adenomatous-like structures with fairly cellular stroma and lymphocytic infiltration.

H. and E. x 150

APPENDIX 1

Age and Histology Summary of Patients taking "Oral Contraceptive Hormones"

Serial Nos.	Biopsy Nos.	Age	Histology Summary
1	0030/70	28	Fibroadenosis (moderate) + epitheliosis (mild).
2	0533/70	19	Florid fibroadenoma (marked) + florid epitheliosis (moderate) + fibrosis (moderate).
3	0110/70	36	Florid epitheliosis (marked) + carcinoma + fibrocystic disease.
4	1121/66	24	Florid fibroadenoma + epitheliosis (marked) + cystic formation.
5	7392/66	43	Fibrosis (marked) + adenosis (mild) + epitheliosis (mild).
6	8362/65) 8386/65)	22	Florid fibroadenoma + florid epitheliosis (moderate) + fibrosis (marked).
7	0812/69	30	Florid fibroadenoma + epitheliosis (mild) + fibrosis (mild).
8	4613/66) 4639/66) 4981/66)	41	Epitheliosis (marked) + florid fibroadenoma + fibroadenosis + carcinoma.
9	9422/69	25	Adenomatous-like fibroadenosis + epitheliosis (moderate) + florid fibroadenosis.
10	0279/69	27	Fibrosis (moderate) + adenosis (mild) + epitheliosis (mild).
11	8253/69	33	Fibroadenosis (moderate) + epitheliosis (mild).
12	9848/69	28	Adenosis (moderate) + epitheliosis (moderate) + carcinoma.
13	2487/70	21	Florid fibroadenoma + epitheliosis (moderate).
14	3293/70	38	Fibrocystic disease + epitheliosis (marked).
15	9540/69	32	Fibroadenosis + fibrosis (moderate) + epitheliosis (mild).
16	2725/69	22	Florid fibroadenoma (moderate) + epitheliosis (mild).
17	2726/69	21	Fibrosis (moderate) + adenosis.
18	1472/69	26	Fibroadenoma + epitheliosis (mild).
19	1471/69	46	Fibroadenosis + fibrosis.

APPENDIX 1 (contd.)

Serial Nos.	Biopsy Nos.	Age	Histology Summary
20	8641/68	30	Fibroadenosis + fibrosis (marked).
21	9227/68	34	Fibroadenosis + fibrosis (moderate) + epitheliosis (mild).
22	7017/68	20	Adenosis + papilloma in small duct.
23	0908/70	20	Florid fibroadenoma + mild epitheliosis.
24	0754/70	23	Florid fibroadenosis + epitheliosis (moderate) + fibrosis.
25	0856/70	25	Adenosis + fibrosis.
26	4711/70	38	Florid fibroadenosis (marked) + epitheliosis (moderate) + carcinoma.
27	1802/70	44	Adenosis (moderate) + epitheliosis (moderate) + fibrosis.
28	3297/70	22	Fibroadenosis + epitheliosis (moderate).
29	4336/70	26	Fibrosis (marked) + adenosis.
30	3447/70	29	Florid fibroadenoma + epitheliosis (marked).
31	2491/70	47	Fibroadenosis + epitheliosis (moderate).
32	0709/70	22	Epitheliosis (marked) + papillomatosis + fibrocystic disease.
33	3049/69	28	Intraductal and invasive carcinoma + fibrosis.
34	1985/69	24	Hyperplastic fibroadenoma + epitheliosis.
35	2509/69	30	Florid fibroadenoma + epitheliosis (marked).
36	0431/69	27	Fibrocystic disease + epitheliosis.
37	0361/69	20	Adenosis + epitheliosis (moderate).
38	0299/69	24	Florid adenosis + epitheliosis.
39	3872/69	30	Fibroadenosis (moderate) + epitheliosis (marked).
40	3444/69	28	Florid adenosis + fibrosis.
41	4955/69	26	Hyperplastic fibroadenoma + epitheliosis.
42	5129/69	43	Fibrocystic disease + epitheliosis (marked).
43	3527/69	37	Fibrocystic disease + moderate epitheliosis.
44	8626/69	29	Fibroadenosis + epitheliosis (mild).
45	5779/70	29	Florid fibroadenoma + epitheliosis (moderate).

APPENDIX 2

Age and Histology Summary of Patients not taking "Hormone Treatment"

Serial Nos.	Biopsy Nos.	Age	Histology Summary
1	5954/65	21	Fibroadenosis + epitheliosis.
2	2959/67	32	Fibroadenosis.
3	7935/67	31	Fibroadenosis + fibrosis.
4	5633/65	23	Adenosis.
5	4644/67	36	Adenosis.
6	1358/68	27	Fibroadenosis + epitheliosis.
7	6523/67	32	Fibroadenosis.
8	8062/66	24	Fibroadenoma.
9	5641/66	19	Fibroadenoma.
10	2767/67	27	Fibroadenoma + adenosis.
11	0474/68	24	Fibroadenoma + focus of adenosis.
12	7894/67	34	Fibroadenoma + adenosis.
13	1130/67	20	Fibroadenoma.
14	8496/66	32	Adenosis + epitheliosis.
15	1182/68	26	Fibroadenosis.
16	2450/67	30	Fibroadenosis.
17	4059/67	47	Fibroadenosis.
18	1336/66	35	Adenosis.
19	9030/67	35	Fibroadenosis + focus of fibroadenoma.
20	0205/67	27	Fibroadenoma.
21	0857/70	37	Fibroadenoma + epitheliosis.
22	4744/66	18	Adenosis + fibrosis.
23	7770/66	24	Fibroadenosis + focus of fibroadenoma.

APPENDIX 2 (contd.)

Serial Nos.	Biopsy Nos.	Age	Histology Summary
24	4963/67	33	Adenosis + focus of fibroadenoma.
25	4170/67	43	Fibrocystic disease + carcinoma.
26	7015/67	40	Fibroadenosis.
27	4684/70	18	Fibroadenoma + fibroadenosis.
28	4525/70	19	Fibroadenosis.
29	1735/70	28	Fibroadenoma.
30	2202/70	19	Fibroadenoma.
31	1992/70	23	Fibroadenoma.
32	3312/70	30	Fibroadenoma.
33	3855/70	29	Fibroadenosis.
34	3801/70	23	Fibroadenosis.
35	3800/70	29	Fibroadenoma.
36	3761/70	17	Fibroadenosis.
37	3346/70	20	Fibroadenoma.
38	3347/70	26	Fibroadenosis.
39	2726/70	21	Fibroadenosis.
40	2487/70	21	Fibroadenoma.
41	2488/70	25	Fibroadenosis.
42	2492/70	29	Fibroadenosis.
43	4603/70	19	Fibroadenosis.
44	4923/70	20	Fibrocystic disease + epitheliosis.
45	0457/69	20	Fibroadenoma.

REFERENCES

ACKERMAN, L.V. and TAYLOR, H.B. (1969). Lesions of the Mammary Gland. 35th Annual Seminar, American Society of Clinical Pathologists, September 19th, Chicago.

ALGIRE, G.H. (1943). J. nat. Cancer Inst., 4, 13.

ALGIRE, G.H. and CHALKLEY, H.W. (1945). J. nat. Cancer Inst., 6, 73.

ALGIRE, G.H., LEGALLAIS, F.Y. and PARK, H.D. (1947). J. nat. Cancer Inst., 8, 53.

ALGIRE, G.H. and LEGALLAIS, F.Y. (1951). J. nat. Cancer Inst., 12, 399.

ALLEN, W. (1969). Cancer, 24, 1137.

ALTURA, B.M. and ZWEIFACH, B.W. (1966). Angiology, 17, 493.

BAKER, P., WEISER, R.S., JUTILA, J., EVANS, C.A. and BLANDAU, R.J. (1962). Ann. New York Acad. Sc., 101, 46.

BERN, H.A. and NANDI, S. (1961). Progr. exp. Tumour Res., 2, 90.

BONSER, G.M., DOSSETT, J.A. and JULL, J.W. (1961). "Human and Experimental Breast Cancer," 1st ed. London: Pitman Medical Publishing Co. Ltd.

BOURA, A.L.A. and GREEN, A.F. (1965). Ann. Rev. Pharmacol., 5, 183.

BRAITHWAITE, J. L. (1958). Brit. J. Cancer, 12, 75.

BROWN, W.H. and PEARCE, L. (1920-21). Proc. Soc. exp. Biol., 18, 201.

BURGESS, E.A. and SYLVÉN, B. (1962). Cancer Res., 22, 581.

CANDY, J. and ABELL, M.R. (1968). J. Amer. med. Ass., 203, 323.

CHANG, C.H. and TREMBLY, B. (1961). Yale J. Biol. Med., 33, 451.

CHARLES, D.J. (1964). J. clin. Path., 17, 205.

CHEATLE, G.L. and CUTLER, N. (1931). Tumours of the breast, p. 112. Arnold, London.

COHN, J.N., LIPTAK, T.E. and FREIS, E.D. (1963). Circulat. Res., 12, 298.

COMAN, D.R. and SHELDON, W.F. (1946). Amer. J. Path., 22, 821.

DAWSON, E.K. (1933). Edinb. med. J., 40, 57.

DRILL, V.A. (1965). Metabolism, 14, 295.

~~EL HASSAN, A.M. and STUART, A.E. (1965). Brit. J. Cancer, 19, 343.~~

EVANS, L. (1968). "Principle of Human Physiology", 14th ed., p. 241. London.

FECHNER, R.E. (1970a). Cancer, 25, 1332.

FECHNER, R.E. (1970b). Amer. J. clin. Path., 53, 857.

FECHNER, R.E. (1970c). Cancer, 26, 1204.

GALLAGER, H.S. and MARTIN, J.E. (1969). Cancer, 24, 1170.

GOLDACRE, R.J. and SYLVÉN, B. (1962). Brit. J. Cancer, 16, 306.

GOLDENBERG, V.E., MOTTET, N.K. and WOLFF, M. (1968). Amer. J. clin. Path., 50, 635. (Abstr.).

GOLDENBERG, V.E., WIEGENSTEIN, L. and MOTTET, N.K. (1968). Amer. J. clin. Path., 49, 52.

GOLDMAN, E.E. (1907). Proc. roy. Soc. Med., 1, 1.

GOODALL, C.M., SANDERS, A.G. and SHUBIK, P. (1965). J. nat. Cancer Inst., 35, 497.

GOODALL, C.M., SANDERS, A.G., SHUBIK, P., FELDMAN, R. and KUNKLER, G. (1964). Fed. Proc., 23, 287.

GREGG, W.I. (1966). New Eng. J. Med., 275, 1138.

GULLINO, P.M. and GRANTHAM, F.H. (1962). J. nat. Cancer Inst., 28, 211.

GULLINO, P.M., CLARK, S.H. and GRANTHAM, F.H. (1964). Cancer Res., 24, 780.

GULLINO, P.M., GRANTHAM, F.H., COURTNEY, H. and LOSONCZY, I. (1967). Cancer Res., 27, 1041.

GUYTON, A.C. (1963). Circulat. Res., 12, 399.

GUYTON, A.C. and COLEMAN, T.G. (1968). Ann. N.Y. Acad. Sci., 150, 537.

HASEGAWA, K. (1934). Gann., 28, 32.

HERTZ, R. (1969). Cancer, 24, 1140.

HERTZ, R. and BAILAR, J.C. (1966). J. Amer. med. Ass., 198, 1000.

HOLTON, P. and RAND, M.J. (1962). Brit. J. Pharmacol., 19, 513.

HORSELEY, J.S., III., ALRICH, E.M., and WRIGHT, C.B. (1969). Ann. Surg., 169, 839.

IDE, A.G., BAKER, N.H. and WARREN, S.L. (1939). Amer. J. Roentgenol., 43, 891.

IIJIMA, N., YAMAMOTO, T., INOUE, K., SOO, S. and MATSUZAWA, A. (1969). Jap. J. exp. Med., 39, 205.

KAHN, R.H. (1964). Endocrinology, 75, 818.

KJELLMER, I. (1964). Acta Physiol. scand., 62, 31.

KLÜVER, J. and BARRERA, E. (1953). J. Neuropath exp. Neurol., 12, 400.

KYRIAKOS, N., KEMPSON, R.L. and KONIKOV, N.F. (1968). Cancer, 22, 99.

LADEGAARD-PEDERSON, H.J. (1970). Circulat. Res., 26, 765.

LAGERGREN, C., LINBOM, Å. and SÖDERBERG, G. (1958). Acta radiol., 49, 441.

LAGERGREN, C., LINBOM, Å. and SÖDERBERG, G. (1960). Acta radiol., 53, 1.

LE GAL, Y. (1961). Amer. Surg., 27, 14.

LENDRUM, A.C., FRASER, D.S., SLIDDERS, W. and HENDERSON, R. (1962). J. clin. Path., 15, 401.

LEWIS, W.H. (1927). Johns Hopk. Hosp. Bull., 41, 156.

LIEN, W.N. and ACKERMAN, N.B. (1970). Surgery, 68, 334.

LINDREGN, A. (1945). Acta path. microbiol. scand., 22, 493.

MALLORY, F.B. (1938). "Pathological Technique". Philadelphia and London.

MARGOLIS, G. and PICKETT, J.P. (1956). Lab. Invest., 5, 459.

MAXWELL, R.A., PLUMMER, A.J., SCHNEIDER, F., POVALSKI, H. and DANIEL, I. (1960). J. Pharmacol. exp. Ther., 128, 22.

McCARTHY, J. (1965). Amer. J. Surg., 110, 720.

McDONALD, D.A. (1968). Ann. Rev. Physiol., 30, 525.

OBER, W.B. (1966). J. clin. Path., 19, 138.

RUBIN, P. and CASARETT, G.W. (1966). Clin. Radiol., 17, 220.

RUBIN, P., CASARETT, G.W., KURCHARA, S.S. and FUJII, M. (1964). Amer. J. Roentgenol., 92, 378.

SANDERS, A.G. (1971). Microvascular Patterns in Tumour. 122nd Meeting, Pathological Society of Great Britain and Ireland, January 8th. The Middlesex Hospital, London.

SCHACHNER, S.H. (1966). New Engl. J. Med., 275, 1138.

SCHEID, P. (1961). Bibl. anat. (Basel), 1, 327.

SHINKAWA, T. (1939). Nagoya J. med. Sci., 13, 263.

SHIPMAN, J.J. (1964). Brit Med. J., 4509, 629.

SHIVAS, A.A. (1955). M.D. Thesis, University of Aberdeen.

SHIVAS, A.A. (1959). J. Path. Bact., 78. 81.

SHIVAS, A.A. and FINLAYSON, N.D.C. (1965). Brit. J. Cancer, 19, 486.

SHIVAS, A.A. and GILLESPIE, W.J. (1969). Brit. J. Cancer, 23, 638.

STARLING, E.H. (1896). J. physiol. (London), 19, 312.

STERN, E. and MICKEY, M.R. (1969). Brit. J. Cancer, 23, 391.

STUART, A.E. and EL HASSAN, A.M. (1964). Brit. J. Cancer, 18, 551.

TANNOCK, I.F. and STEEL, G.G. (1969). J. nat. Cancer Inst., 42, 771.

* STUART, A.E. and EL HASSAN, A.M. (1965). Brit. J. Cancer, 19, 343.

TANNOCK, I.F. and STEEL, G.G. (1970). J. nat. Cancer Inst., 45, 123.

TAYLOR, H.B., IREY, N.S. and NORRIS, H.J. (1967). J. Amer. med. Ass., 202, 637.

THOMLINSON, R.H. and GRAY, L.H. (1955). Brit. J. Cancer, 9, 539.

THUNOLD, S. (1968). Acta Path. microbiol. scand., 72, 542.

WARBURG, O. (1956). Science, 123, 309.

WARREN, B.A. (1967). Bibl. anat. (Basel), 9, 412.

WATERS, H.G. and GREEN, J.A. (1959). Cancer Res., 19, 326.

WEISBURGER, J.H., WEISBURGER, E.K., CRISWALD, D.P.J.R. and CASEY, A.E. (1968). Life sci., 7, 259.

WIEDERHIELM, C.A. (1968). J. gen. Physiol., 52, 29.

WILLIAMS, R.G. (1951). Cancer Res., 11, 139.

WILLIS, R.A. (1967). "Pathology of Tumours", 4th ed. London: Butterworth.

WITTE, S. and GOLDENBERG, D.M. (1967). Bibl. anat. (Basel), 9, 396.

YAFFEE, H.S. (1966). New Engl. J. Med., 274, 1446.

YOUNG, J.S. and GRIFFITH, H.O. (1950). J. Path. Bact., 62, 293.

YOUNG, J.S., LUMSDEN, C.E. and STALKER, A.L. (1950). J. Path. Bact., 62, 313.

YOUNGER, J.S. and ALGIRE, G.H. (1949). J. nat. Cancer Inst., 10, 565.